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AUSTRALASIAN ANNALS OF MEDICINE

Journal of The Royal Australasian College of Physicians

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"HÆMOPHILIA": A SYNDROME UNDER REVIEW

INVESTIGATIONS OF ALPHA AND BETA PROTHROMBOPLASTIN DEFICIENCIES[1]

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CLOTTING of blood is the result of an intricate chain of reactions which requires several components and accessory factors. Thromboplastin can be considered the substance which sets off the clotting process. Although hæmorrhagic tendencies due to inhibitors of the thromboplastin complex have been known for some time, until a few years ago hæmophilia was the only condition recognized clinically as being caused by a derangement of the thromboplastin complex. The first indication that more than one type of deficiency could produce a hæmophilic syndrome came from the occasional observation that blood specimens taken from two apparent hæmophiliacs, each of whom had a prolonged whole blood clotting time, when mixed "had a coagulant action nearly as effective as normal blood" (Pavlovsky, 1947). Further, it was noted that the anti-hæmophilic fraction isolated from normal human plasma was of temporary benefit in the treatment of some patients, but of no avail to others. Aggeler *et alii* (1952) have recognized clearly that bleeding tendencies, believed to be hæmophilia on clinical grounds, can be caused by deficiency of either of two chemically different factors. Additional clinical reports and laboratory findings related to thromboplastin deficiencies have been made by Koller *et alii* (1950), Biggs *et alii* (1952), Schulman and Smith (1952), Van Creveld and Paulssen (1953), Poole (1953), Lewis and Ferguson (1953), Rosenthal *et alii* (1953), Fantl and Sawers (1954).

[1] Received on June 30, 1954.

[2] Part of the expenses of this investigation were defrayed by a grant from the National Health and Medical Research Council, Commonwealth of Australia.

[3] R. B. McComas Research Fellow.

Clinical symptoms and the results of laboratory tests usually correlate well in severe deficiencies, but in mild cases it is at times difficult to decide whether hæmorrhages are due to a slight deficiency of a clotting component or to other causes. Therefore it is essential to assess the deficiency quantitatively.

In the following study an attempt has been made to provide a quantitative basis for the assay of blood thromboplastin activity, and results of laboratory tests are compared with clinical findings.

TERMINOLOGY

The clinical symptoms of hæmophilia and of the hæmorrhagic conditions resembling it are identical; therefore terminology based on symptoms is not possible. Other hæmorrhagic diseases due to a deficiency of a coagulation factor are named after the factor concerned—for example, hypoprothrombinæmia. Although the compounds required for the formation of blood thromboplastin have not been isolated in pure form, sufficient separation and characterization from the other plasma constituents have been obtained to justify a nomenclature based on biochemical properties. The two major components contributing to plasma thromboplastin will be called α and β prothromboplastin; α prothromboplastin is synonymous with anti-hæmophilic globulin (Lewis *et alii*, 1946) and anti-hæmophilic factor (AHF—Brinkhous *et alii*, 1951). Synonyms for β prothromboplastin are plasma thromboplastin component (PTC—Aggeler *et alii*, 1952) or Christmas factor (Biggs *et alii*, 1952).[1] Hæmophilia becomes then α prothromboplastin deficiency or α prothromboplastinopenia, and

[1] For other suggestions, see *Blood* (1954), **9**, 246.

PTC deficiency or Christmas disease becomes β prothromboplastin deficiency. If in future more components are isolated which contribute to plasma thromboplastin like the suggested PTC antecedent (Rosenthal *et alii*, 1953), the next letter in the Greek alphabet should be used. Further, if factors are found which influence the activity of the α component, they should be called, for example, α cothromboplastin, or if inhibitors are isolated they should be called α antiprothromboplastin *et cetera*.

EXPERIMENTAL INVESTIGATION

For this investigation blood was obtained from normal subjects and from 43 patients who clinically were considered to be " bleeders ".

Venous blood was withdrawn, preferably in the direction of blood flow. Two syringes were used. The contents of the first were discarded, and from the second blood was obtained for all tests. At a temperature of 37° C. whole blood clotting time was determined usually with 1·5 millilitres, except where stated, in " Pyrex " glass tubes, and in similar tubes coated with silicone according to the technique of Jacques *et alii* (1946).

Oxalated or citrated blood was obtained by mixing one volume of 0·1M sodium oxalate solution or one volume of 0·13M trisodium citrate solution with nine volumes of blood.

Platelet-poor plasma was obtained, after centrifugation of blood at 2300 g, at 5° C. for twenty minutes.

Capillary blood was obtained from a pricked finger or heel; 0·18 millilitre was drawn into a pipette containing 0·02 millilitre of 0·1M sodium oxalate solution and discharged into 0·8 millilitre of 0·15M sodium chloride solution at 37° C.

Prothrombin-free plasma was prepared by adding with frequent stirring 20 milligrammes of freshly prepared barium sulphate per millilitre of oxalated plasma at 5° C. for thirty minutes. The clear plasma will be referred to as Ba-plasma.

Stored plasma refers to oxalated plasma, to which had been added " Merthiolate " to a final concentration of 10^{-4}. The specimens were kept in test tubes plugged with cotton wool at room temperature for at least ten days. All dilutions of blood and blood derivatives were made with 0·15M sodium chloride solution.

Recalcification time was determined with 0·2 millilitre of plasma and an equal volume of 0·025M calcium chloride solution at 37° C.

Prothrombin determinations were carried out according to the method of Quick (1951) in plasma and in serum. In the case of serum the specimen was diluted with an equal volume of Ba-plasma. The absolute amount of prothrombin was assayed by the procedure of Fantl (1954*a*). The determination of blood thromboplastin activity was carried out indirectly by measuring the rate of formation and yield of thrombin in diluted blood in the presence of pyrocatechol, which acts as a thrombin stabilizer.

Venous oxalated blood (0·5 millilitre) is added to two millilitres of 0·15M sodium chloride solution immediately after blood collection; 0·2 millilitre of this dilution is mixed with a solution consisting of 0·6 millilitre of 0·025M calcium chloride solution, 0·7 millilitre of 0·15M sodium chloride solution and 0·3 millilitre of 0·18M pyrocatechol solution in a " Veronal " buffer, at pH 7·4 and at a reaction temperature of 28° C. At intervals beginning ten minutes from the start, 0·2 millilitre of the reaction mixture is removed and blown into a mixture of 0·2 millilitre of Ba-plasma and 0·05 millilitre of 0·18M pyrocatechol solution in a " Veronal " buffer at pH 7·4. Determinations of the clotting times are carried out until two successive readings are identical.

The details and the theoretical aspects of this technique have been given by Fantl (1954*b*). The results of the prothromboplastin assay are expressed in units of thrombin.

In the blood of the patients to be discussed, it was ascertained that prothrombin was present in normal concentration and activity. As the results of Quick's test and those obtained by an absolute assay agreed, prothrombin accelerators were also present in normal concentrations. The determination of fibrinogen and the number of thrombocytes indicated that these two components were present in normal amounts. Inhibitors of blood coagulation were not detectable. Most of the patients have been under observation for several years, and the results of repeated tests did not vary by more than the experimental error inherent in the techniques employed.

Whole Blood Clotting Time in Thromboplastin Deficiencies

The scattergram in Figure I gives the whole blood clotting time obtained from specimens from 37 patients. Each result given in Figure I is the average of three or four determinations.

Under our experimental conditions, the clotting time of blood from 33 normal donors obtained in glass varied between nine and nineteen minutes. The corresponding figures in silicone were between thirty and ninety minutes. It appears that the results given in Figure I can be divided into two main groups. In 21 results the whole blood clotting time in glass was either normal or moderately pro-

longed, ranging from fifteen to thirty-five minutes, and the whole blood clotting time in silicone was between ninety-five and four hundred minutes. The other group comprises 16 results with whole blood clotting times

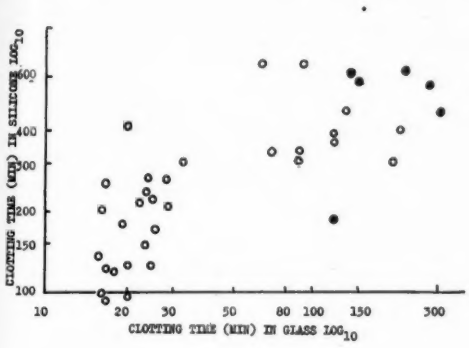

FIGURE I

Whole blood clotting times of patients with α prothromboplastin deficiencies are indicated by open circles, those of patients with β deficiencies by solid circles.

between seventy and three hundred minutes in glass. The corresponding figures in silicone are one hundred and eighty and six hundred minutes. If the individual results were plotted, the results would spread more uniformly over the whole area; but the average figures show the interesting phenomenon that there is a gap in the clotting times between forty and seventy minutes. It will be noted that in the group of α prothromboplastin deficiency, variations of whole blood clotting time from normal to grossly delayed results were obtained, whereas in all the six cases of β prothromboplastin deficiency the whole blood clotting time was grossly delayed.

TABLE I

Influence of 2% of Normal Blood on the Clotting Time of α and β Prothromboplastin Deficient Blood[1]

Type of Deficiency	Clotting Time in Minutes	
	Without Addition	With Addition of 2% Normal Blood
α	90	16
α	90	12
α	70	12
α	100	13
β	300	13

[1] The blood specimens were taken with a silicone-coated syringe and kept in a silicone-coated test tube in an ice bath. A few minutes afterwards normal blood was obtained in a similar manner, and 0·04 millilitre of the latter was added to 1·96 millilitres of the patient's blood with silicone-coated pipettes. After gentle and careful mixing the specimens were transferred to a water bath at 37° C.

The relationship between clotting time and degree of deficiency was investigated with mixtures of blood specimens with a clotting time longer than seventy minutes and normal blood. The results are given in Table I.

From the results given in Table I, it is apparent that the addition of 2% of normal blood to blood specimens with a clotting time longer than seventy minutes is sufficient to reduce the clotting time to a normal level. These results are taken to mean that complete absence of either α or β prothromboplastin gives grossly delayed clotting time, whereas the clotting times in cases in which the blood thromboplastin concentration is quite low are not very different from normal figures.

Determination of the Coagulation Defect

In order to establish which component of the thromboplastin complex was deficient, the following tests were carried out, based on the knowledge that Ba-plasma is a source of α prothromboplastin and is almost free of the β precursor, whilst with stored plasma the reverse is true (White *et alii*, 1953; Biggs *et alii*, 1952). It is therefore possible to establish the diagnosis of α prothromboplastin deficiency when fresh Ba-plasma shortens the clotting time of the patient's plasma, and a prothrombin accelerator deficiency is excluded. The clotting time of plasma deficient in α prothromboplastin is not reduced by the addition of stored plasma. On the other hand, in bleeding tendencies due to β prothromboplastin deficiency, the clotting time of plasma is shortened by the addition of stored plasma, and little reduction by Ba-plasma is usually observed. The results of tests in which Ba-plasma is used as well as stored plasma are given in Tables II and III.

TABLE II

Plasma Recalcification Times in α Prothromboplastin Deficiencies (Minutes)

Name[1]	Plasma	Plasma *plus*			
		Ba-Plasma		Stored Plasma	
		50%	10%	50%	10%
GI-1 ..	160	7	7·5	50	—
GF-1 ..	65	8·5	—	44	—
GH-1 ..	60	6·5	7·5	34	—
GA-1 ..	53	8	—	—	44
GD-1 ..	55	9·5	—	32	—
GG-1 ..	41	6·5	6·5	50	—
GB-1 ..	37	7	7·5	22	—
GE-1 ..	28	7	8·0	—	30

[1] A pedigree is identified by a two-letter code. A patient is identified by this code and an additional numeral suffix.

From the results given in Table II it is evident that the patients listed there are deficient in α prothromboplastin, and the results given in Table III indicate that the patients are suffering from β prothromboplastin deficiency. It is interesting to note that the addition of approximately 10% of Ba-plasma in the case of α prothromboplastinopenia and 10% of stored plasma in the case of the β form was in most cases sufficient to shorten the plasma recalcification time to normal values.

TABLE III

Plasma Recalcification Times in β Prothromboplastin Deficiencies (Minutes)

Name	Plasma	Plasma *plus*		
		Stored Plasma		Ba-Plasma
		50%	10%	50%
MA-2	240	10	8	84
MD-2	150	11	12	180
MD-1	110	8·5	9·5	67
MB-1	>60	8	7·5	180
MC-1	22	7	5	16

It should be emphasized that in our experience normal human serum obtained twenty-four hours after coagulation was not suitable for diagnostic purposes in experiments similar to those set out in Tables II and III, because it reduced the coagulation time of normal plasma as well as the plasma of the patients. Apparently small amounts of α thromboplastin and thromboplastin activators were present.

Determination of Degree of Prothromboplastin Deficiency

The amount of Ba-plasma in α prothromboplastinopenia or the amount of stored plasma in β prothromboplastinopenia necessary to produce a normal recalcification time gives some indication of the severity of the deficiency of the particular factor, but does not give a quantitative measure of deficiency. A further indication of a prothromboplastin deficiency is obtained from the concentration of prothrombin in serum. Brinkhous (1939) has shown that prothrombin disappears rapidly from clotting normal blood. Under our experimental conditions sixty to one hundred and fifty minutes after clotting between 0 and 12% of plasma prothrombin was present in serum in normal cases. Higher figures indicated thromboplastin deficiencies. In blood specimens from patients with blood clotting times longer than thirty-five minutes, the prothrombin concentration in serum was always greater than 20% of that of the plasma. Therefore the result was quite clear-cut, but with clotting times approaching the normal, serum prothrombin levels falling within the normal range have been observed, as will be seen from results given in Table IV.

TABLE IV

Serum Prothrombin in Partial α Prothromboplastin Deficiencies

Name	Whole Blood Clotting Time, Two Millilitres (Minutes)	Serum Prothrombin 65 Minutes After Clotting		
		Quick (Seconds)	Prothrombin Assay (Units)	Percentage of Plasma Prothrombin
AG-2 ..	26	21	63	10
AA-2 ..	15	29	42	7
AA-1 ..	15·5	23	65	10
AA-3 ..	15·5	25	63	11
Normal (22 subjects)	9 to 19	>20	0 to 70	0 to 12

From the results given in Table IV it is apparent that the laboratory diagnosis in these cases was uncertain, and it may be added that one of the listed patients bled excessively after tooth extractions, which necessitated frequent blood transfusions. The application of more sensitive laboratory tests seemed desirable to establish the deficiency.

Determination of Blood Thromboplastin Activity by the Rate of Formation and Yield of Thrombin

The ability to produce thrombin in diluted blood was used as a measure of thromboplastin activity. If blood clots, thrombin is formed and destroyed. The amount of thrombin which is present in clotting blood at a given moment depends, therefore, on the balance between formation and destruction. Under these conditions it is obviously not possible to obtain quantitative results. Recently it was shown that phenols have the property of delaying the destruction of thrombin by plasma (Fantl, 1954*a*). In the presence of phenols it should be possible to assess quantitatively the ability of a system to form thrombin. With this device the relationship between thromboplastin activity and rate of formation and yield of thrombin was investigated. By the use of this technique, one millilitre of normal blood produces 160 to 240 units of thrombin during an incubation time of fourteen to nineteen minutes (Fantl, 1954*b*).

Representative results obtained by this technique are illustrated in Figure II. (The curves in the various figures are distinguished by being intersected by solid circles, open circles, triangles, rectangles, crosses *et cetera*).

From the results shown in Figure II it will be noted that the patient's diluted blood did not produce any thrombin during sixty minutes' incubation (solid circles). The addition of 12·5% serum had very little influence on the formation of thrombin (triangles); but the addition of 12·5% Ba-plasma (rectangles) or

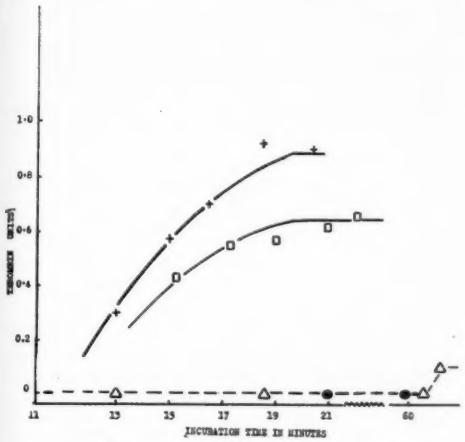

FIGURE II

Rate of formation and yield of thrombin in mixtures of normal and α prothromboplastin deficient blood. (i) Patient GE-1; the determination of blood thromboplastin activity was carried out with 0·2 millilitre of a 20% dilution of oxalated venous blood as described in the text (solid circles). (ii) The same as (i) with the addition of 25% of normal blood (crosses). (iii) The same as (i) with the addition of 12·5% of serum (triangles). (iv) The same as (i) with the addition of 12·5% of Ba-plasma (open rectangles). Results are given in units of thrombin for 0·2 millilitre of reaction mixture.

25% of normal blood (crosses) produced normal amounts of thrombin in normal time. These observations indicate that GE-1 is suffering from a complete α-prothromboplastin deficiency. The steeper curve in the case of mixtures of the patient's and normal blood is due to the presence of 25% more prothrombin than in the other instances. Similar results to those observed in Figure II have been obtained with the blood of seven more patients.

In contrast to the case just described, Figure III is representative of a complete β prothromboplastin deficiency.

From the results given in Figure III it will be noted that the patient's diluted blood did not produce any thrombin during sixty minutes' incubation (solid circles). In contrast to blood deficient in α prothromboplastin, this patient's blood produced thrombin after the addition of 12·5% serum or stored plasma (rectangles and open circles), but not with Ba-plasma (crosses). These observations indicate that MA-2 is suffering from a complete β prothromboplastin deficiency. Similar results to those observed in Figure III have been encountered in five more cases.

Determinations of the rate of formation and yield of thrombin were carried out in the diluted blood of 40 patients, and the results are given in Figure IV.

It will be noted from the results given in Figure IV that the time for maximum thrombin formation varied between twenty and a half and more than fifty minutes; eight patients with an α prothromboplastin deficiency and all six patients with a β deficiency produced no thrombin or traces only. They are considered to have a complete absence of the particular thromboplastin precursor in their

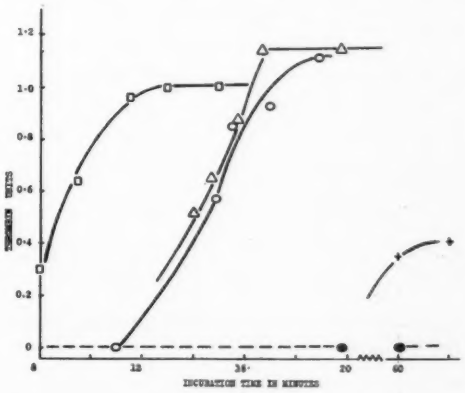

FIGURE III

Rate of formation and yield of thrombin in mixtures of normal and β prothromboplastin deficient blood. (i) Patient MA-2; 0·2 millilitre of 20% oxalated venous blood (solid circles). (ii) The same as (i) with the addition of 12·5% of serum (open rectangles). (iii) The same as (i) with the addition of 12·5% of normal oxalated plasma (triangles). (iv) The same as (i) with the addition of 12·5% of stored oxalated plasma (open circles). (v) The same as (i) with the addition of 12·5% of Ba-plasma (crosses). The experimental conditions were similar to those given in Figure II (i).

circulation. With regard to the remaining 26 patients with α prothromboplastin deficiency, it appears that they have a partial deficiency of the α thromboplastin precursor. Their blood can form a maximum amount of thrombin in periods between twenty and a half and fifty minutes. It is noteworthy that 14 of these patients, despite the delayed thrombin formation rate, eventually produce a normal yield of thrombin.

The relationship between the rate of formation and total yield of thrombin in a group of partial α prothromboplastin deficiencies was also investigated before and after the addition of

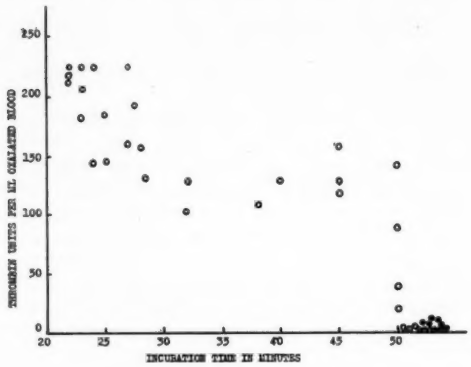

FIGURE IV

Rate of formation and total yield of thrombin in diluted venous blood deficient in α or β prothromboplastin. In the reaction mixture 0·2 millilitre of a 20% dilution of oxalated venous blood was used. α prothromboplastin deficiency is indicated by open circles and β prothromboplastin deficiency by solid circles. The experimental conditions were the same as those given in Figure II (i).

diluted normal blood to the patient's diluted blood. Results of such experiments are given in Table V.

A comparison between the results obtained from the patient's blood and those obtained from normal blood indicates that the yield of thrombin obtainable in the five cases (Table V) was similar to that of normal blood within experimental error of the technique, which is approximately 10%, although the reaction time to obtain maximum thrombin formation was prolonged. With an increase in the reaction time required for maximum thrombin formation, as occurred in AN-1, the thrombin yield did not reach a normal value. Shortening of the reaction time could be obtained in each case after the addition of between 10% and 25% of normal blood. The results given in Figures II, III and IV and Table V show that α prothromboplastin deficiencies exist of degrees varying from complete absence to minor deficiency.

TABLE V

Thrombin Formation of Diluted α Prothromboplastin Deficient Blood

Name	Maximum Thrombin Formation				Thrombin Yield Without Addition as Percentage of Yield After Addition
	Without Addition		After Addition of 25% Normal Blood		
	Minutes	Units	Minutes	Units	
AG-2	23	198	17	225	88
AP-1	23	207	14	189	109
AD-1	25	216	16	207	104
AJ-1	28	171	15	225	76
AO-1	32	164	17	198	82
AN-1*[1]	45	92 to 128	22[1]	174	53 to 73
Normal	14 to 19	160 to 240	—	—	100

[1] In the reaction mixture 0·2 millilitre of 20% venous oxalated blood was used. To AN-1* blood, 10% normal blood was added. Experimental conditions were similar to those given in Figure II (1).

An Explanation of the Defect in α and β Prothromboplastinopenias

The possibilities that the defect in the hæmorrhagic conditions under discussion might be due to a deficiency or to the presence of inhibitors of coagulation and perhaps to both, and further, whether the mechanism of blood thromboplastin activity was of stoicheiometric or of enzymatic nature, were investigated. It seemed that the study of the rate of formation and yield of thrombin in diluted blood in the presence of pyrocatechol might provide an insight into these problems. The behaviour of blood specimens taken from what appeared to be a moderately severe deficiency seemed particularly interesting. These specimens could produce normal amounts of thrombin, although the time for maximum thrombin formation was delayed. Several types of experiments were carried out to find the most likely explanation for these observations. In the one the rate of thrombin formation and thrombin yield was measured in different concentrations of both normal and abnormal blood specimens. The results of such experiments are given in Figure V.

The results plotted in Figure V were obtained from a patient whose blood clotting time was twenty-three minutes in glass and three hundred minutes in silicone. The deficiency could be corrected by the addition of Baplasma, but not stored plasma, to the patient's blood. He had therefore an α prothromboplastin deficiency. The results given in Figure V indicate that 0·06 millilitre of the patient's blood produced 0·7 unit of thrombin in twenty-five minutes (open rectangles). This amount of thrombin is produced by 0·035 millilitre of normal blood in less than twenty minutes. This might indicate a deficiency of approximately 50% of α prothromboplastin in the patient's blood. However, a comparison with the results obtained with 0·03 millilitre (crosses) indicates that normal blood still produced thrombin in a shorter time than twice the

volume of the patient's blood. It should be pointed out that the discrepancy is in fact greater than is indicated by the curves, because of the greater dilutions of normal blood than the patient's blood. These results show that the production of a fraction of the potential thrombin yield does not correspond to a similar reduction in α prothromboplastin concentration. To obtain more information on this point, in another series of experiments the following system was used for a study of thrombin formation. Human oxalated stored plasma was used as a source of β prothromboplastin and prothrombin. Fresh Ba-plasma or fractions

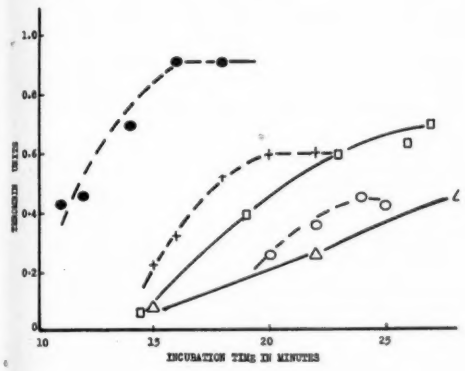

FIGURE V

Comparison between normal and α prothromboplastin deficient blood with regard to rate of formation and yield of thrombin. Patient AJ-1; 0·06 millilitre of oxalated venous blood, open rectangles; 0·04 millilitre, triangles. Normal oxalated venous blood: (i) 0·02 millilitre (open circles); (ii) 0·03 millilitre (crosses); (iii) 0·04 millilitre (solid circles). The composition of the reaction mixture was the same as that in Figure II (i).

made from it were used as a source of α prothromboplastin and prothrombin accelerator. Thrombocytes were added to the mixture, and recalcification in the presence of pyrocatechol was carried out. The results are given in Table VI.

The results given in Table VI indicate that, given a constant amount of prothrombin, prothrombin accelerator and thromboyctes, the concentration of α prothromboplastin can vary between 100% and 25% without influencing the yield of thrombin; but the time to produce the maximum yield of thrombin is delayed according to the dilution.

If α prothromboplastin reacts in stoicheiometric amounts, one would expect that a material reduction would produce a corresponding reduction in the yield of thrombin. Since this is not the case, the results are interpreted to mean that α prothromboplastin takes part in an enzymatic reaction for conversion of prothrombin to thrombin.

In order to overcome the objection that the results obtained with diluted blood in the presence of pyrocatechol are unphysiological and give erroneous results, experiments were carried out in which dilution of blood and the addition of chemicals were avoided. Specimens

TABLE VI

Influence of Varying Amounts of α Prothromboplastin on the Rate of Formation and Yield of Thrombin

Experiment[1]	Type of Prothromboplastin (Millilitre of Plasma)		Thrombocyte Preparation (Millilitres)	Maximum Thrombin Formation	
	β	α		Minutes	Units
I	0·02	0·02	0·1	23	0·62
	0·02	0·01	0·1	26	0·62
	0·02	0·0	0·1	120	0·1
II	0·02	0·02	0·1	23	0·66
	0·02	0·01	0·1	36	0·64
	0·02	0·005	0·1	42	0·64
	0·02	0·002	0·1	78	0·53
	0·02	0·0008	0·1	94	0·35
III	0·02	0·02	0·1	26	0·57
	0·02	0·01	0·1	28	0·66
	0·02	0·005*	0·1	40	0·57
	0·02	0·003**	0·1	52	0·52

[1] In Experiment I, plasma was stored for one month. The thrombocyte preparation (300×10^3 per cubic millimetre), one day old, was kept at +6° C. The composition of the reaction mixture was similar to that given for the determination of thromboplastin activity.

In Experiment II, the thrombocyte preparation was the same as in Experiment I; it was kept at —15° C. for five days. Other conditions were similar to those of Experiment I.

In Experiment III, the thrombocyte preparation was the same as in Experiment I; it was kept at —15° C. for seven days. The figures "0·005*" and "0·003**" indicate that part of the α prothromboplastin was removed by ethanol precipitation at —5° C. in the first case to a final ethanol concentration of 15% volume for volume and in the second case to 24% volume for volume. Other conditions were similar to those in Experiment I. The figures in the α prothromboplastin column are given in arbitrary potencies.

of venous blood were mixed with normal compatible blood in varying proportions. One hundred and fifty minutes after clotting, determinations of prothrombin in the sera were carried out by the assay procedure (Figure VI) and by Quick's procedure (Figure VII).

According to the results given in Figure VI, it is apparent that in two instances of α prothromboplastin deficiency (half open circles, open rectangles) the addition of 25% to 30% normal blood was required to produce a normal serum prothrombin level, whereas in the other four instances of α or β prothromboplastin deficiency the addition of approximately 10% of normal blood was sufficient to correct the defect. The results of serum prothrombin times are given in Figure VII.

The results given in Figure VII should be similar to those given in Figure VI. In general the same conclusions as those from the prothrombin assays (Figure VI) can be drawn. However, the spread of results is far greater than in the assay procedure. The reason is that the sera contained apparently differing

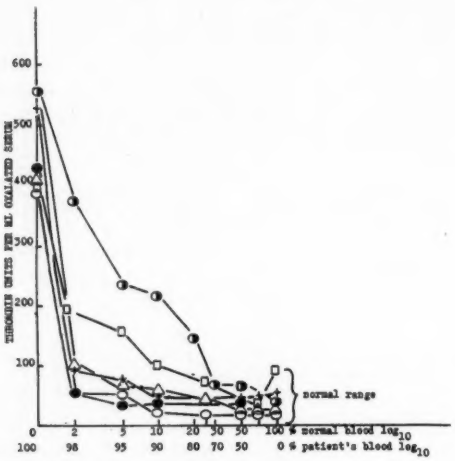

FIGURE VI

Serum prothrombin from mixtures of normal and prothromboplastin deficient blood. Experimental procedure similar to that given in Table I; 150 minutes after clotting at 37° C., 0·22 millilitre of 0·1M sodium oxalate solution was added and mixed with the serum. Prothrombin assays were carried out with the addition of 10% of Ba-plasma. Results are expressed as units of thrombin per millilitre of oxalated serum. The result obtained with β prothromboplastin deficient blood is represented graphically with solid circles. The five other curves were obtained with α prothromboplastin deficient blood. The figures under the abscissa indicate the percentage of normal and patient's blood in the mixture.

amounts of thromboplastin activators, which affect the prothrombin clotting time but have no influence on the absolute amount of prothrombin. Simultaneously with the experiments reported in Figures VI and VII, in which undiluted blood was used, the determination of the rate of formation and yield of thrombin was carried out in diluted blood in the presence of pyrocatechol. Results of a representative experiment are given in Figure VIII.

The results given in Figure VIII show that 0·04 millilitre of patient's blood did not form any thrombin during the period of the observation (interrupted line), and that the addition of approximately 12·5% of normal blood produced a normal rate of formation and yield of thrombin (open rectangles). This was not increased by the addition of 25% normal blood (triangles). The steeper curve is due to the fact that 0·05 millilitre of blood was present in the reaction mixture. On the other hand, the addition of between 1·5% and 6% of normal blood to the patient's blood (open circles, solid circles and crosses) corrected the patient's deficiency only partly, as was indicated by a delayed rate of thrombin formation and also a reduced yield of thrombin, which, however, was still 75% of the potential thrombin yield when 1·5% of normal blood was added. In this experiment, which was carried out with diluted blood, the addition of approximately 12·5% of normal blood was required, whereas in the experiments given in Figure VII, in which undiluted blood was used, between 5% and 10% of normal blood was required to correct the defect. These results would indicate the validity of the prothromboplastin assay.

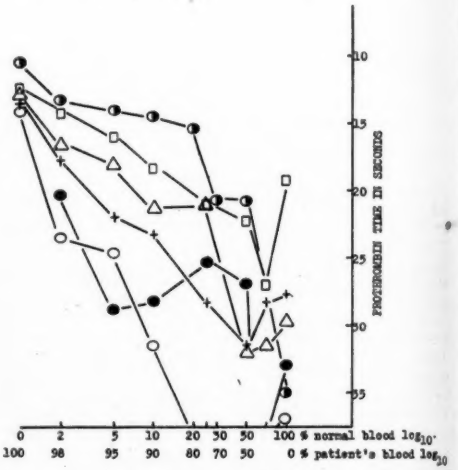

FIGURE VII

The blood specimens were the same as those in Figure VI. Prothrombin determinations were carried out with 0·05 millilitre of oxalated serum and 0·05 millilitre of Ba-plasma.

Comparison between Venous and Capillary Blood with Regard to Rate of Formation and Yield of Thrombin

In order to obtain information regarding the efficacy of treatment of hæmorrhagic conditions, it is desirable to carry out frequent determinations of blood coagulation activity. For this reason capillary blood was investigated. It has been shown previously that the rate of thrombin formation is faster in normal capillary blood than in venous blood, but the yield of thrombin is identical in both instances (Fantl,

1954*b*). In the case of a partial thromboplastin deficiency, examination of capillary blood would lead to erroneous figures, but in the complete absence of the one or the other prothromboplastin precursors results obtained from capillary blood might still be valid. The

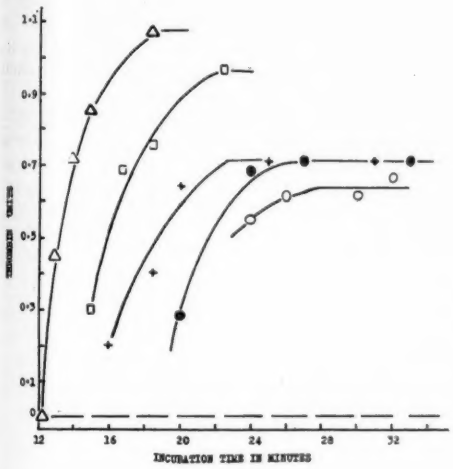

FIGURE VIII

Rate of formation and yield of thrombin in mixtures of normal and α prothromboplastin deficient blood. The blood specimen was the same as that used in Figure VI (crosses). To 0·45 millilitre of venous blood was added 0·5 millilitre of 0·1M sodium oxalate solution. A 20% dilution of the oxalated blood was prepared and 0·2 millilitre was used (interrupted line). In the other experiments normal oxalated blood diluted to the required strength was added to 0·2 millilitre of the patient's diluted blood. Additions were made of 1·5% of normal blood (open circles:, 3% of normal blood (solid circles), 6% of normal blood (crosses), 12·5% of normal blood (open rectangles) and 25% of normal blood (triangles). The composition of the reaction mixture was the same as that given in Figure II (i).

results obtained from venous and capillary blood taken from a patient with complete β prothromboplastin deficiency are given in Figure IX.

The results given in Figure IX show that venous blood did not produce any thrombin during an incubation time of eighty-five minutes (solid circles). Addition of 2% normal serum to diluted venous blood corrected the deficiency (crosses). In contrast to venous blood, capillary blood showed normal behaviour (open circles). These experiments indicate that despite the existence of a complete β prothromboplastin deficiency in blood, the tissues supplied adequate amounts of thromboplastin for normal thrombin formation. This is in marked contrast to complete α prothromboplastin deficiency, in which capillary blood behaves similarly to venous blood.

Comparison between Activity of α or β Prothromboplastin In Vitro and In Vivo

The reported experiments have shown that the rate of formation of thrombin determined by the addition of normal blood or blood derivatives (plasma, Ba-plasma, stored plasma, or serum) to patient's blood assisted in establishing the diagnosis. Further, from the volume of additions required to correct the deficiency *in vitro*, the degree of the defect could be determined. On several occasions the patients had to undergo tooth extractions, and in one case of β prothromboplastin deficiency major surgical interference was necessary because of a hæmorrhagic cyst. It was thought desirable

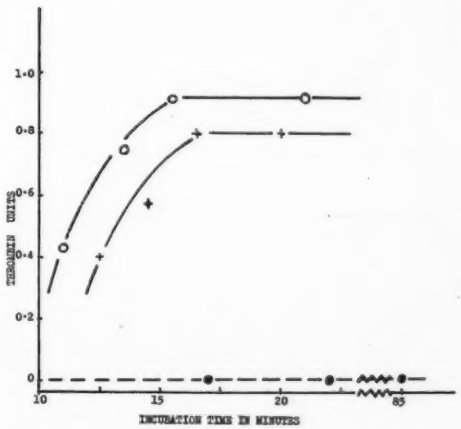

FIGURE IX

Comparison between rate of formation and yield of thrombin in capillary and venous blood devoid of β prothromboplastin. In each case 0·2 millilitre of a 20% dilution of oxalated blood was used. Venous blood is represented by solid circles, capillary blood by open circles and venous blood with 2% of serum by crosses. The other experimental conditions were the same as those given in Figure II (i).

to keep the patients in a good hæmostatic condition, both before and after operation. It was assumed that the blood or plasma addition sufficient *in vitro* to obtain thrombin formation normal in time and yield would also be sufficient for hæmostasis if a corresponding volume was introduced into the circulation. The rate of thrombin formation was determined on the patients' diluted blood, before and after the blood product was transfused.

Influence of Fresh Citrated Plasma on the Rate of Formation and Yield of Thrombin In Vitro and In Vivo on Blood Deficient in α Prothromboplastin

Citrated plasma four hours after collection of blood from normal donors was used. Patient AN-1, aged twenty-two years, weight 90 kilograms, with an assumed blood volume of 6·5 litres, was given 750 millilitres of citrated plasma corresponding to 700 millilitres of native plasma by intravenous transfusion during thirty-five minutes. The results of blood examinations are given in Figure X.

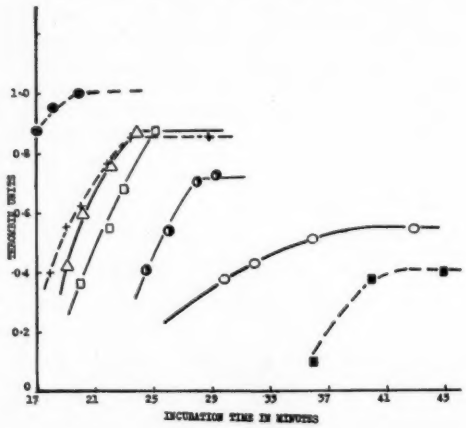

FIGURE X

Influence of fresh citrated plasma on the rate of formation and yield of thrombin *in vitro* in blood deficient in α prothromboplastin. In each case 0·2 millilitre of 20% oxalated blood was used. (i) Patient's blood before transfusion (solid rectangles). (ii) The same as (i) with the addition of 10% of donor's plasma (solid circles). (iii) The same as (i) with the addition of 5% of donor's plasma (crosses). (iv) Patient's blood ten minutes after transfusion (triangles); (v) three hours after transfusion (open rectangles); (vi) six hours after transfusion (half open circles); (vii) twenty-one hours after transfusion (open circles). The composition of the reaction mixture was the same as that given in Figure II (i).

It will be noted from the results given in Figure X that the patient's diluted blood produced 0·4 unit of thrombin per 0·0045 millilitre of blood during forty-five minutes' incubation (solid rectangles). The addition of 10% normal citrated plasma resulted in a normal rate of formation and yield of thrombin (solid circles). Despite the fact that more than 10% of the patient's blood volume was added by the transfusion, ten minutes later—a period which is considered to be adequate for homogeneous distribution throughout the blood space (Gibson and Evans, 1937)—the venous blood contained approximately 5% α prothromboplastin activity (triangles). This concentration was maintained for three hours after the transfusion (open rectangles). But six hours and later after the transfusion, the

TABLE VII

Serum Prothrombin Before and After Plasma Transfusion in α Prothromboplastin Deficient Blood

Time	Whole Blood Clotting Time, Two Millilitres) (Minutes)	Serum Prothrombin 65 Minutes After Clotting		
		Quick (Seconds)	Prothrombin Assay (Units)	Percentage of Plasma Prothrombin
Before transfusion	35	17	432	70
Ten minutes after transfusion ..	19	28	40	6
Three hours after transfusion ..	22	25	80	12
Six hours after transfusion ..	30	20	180	29
Twenty-one hours after transfusion	35	18	210	32

α prothromboplastin activity of the venous blood fell to levels considerably lower than those required for normal hæmostasis. Simultaneously with the results shown in Figure X,

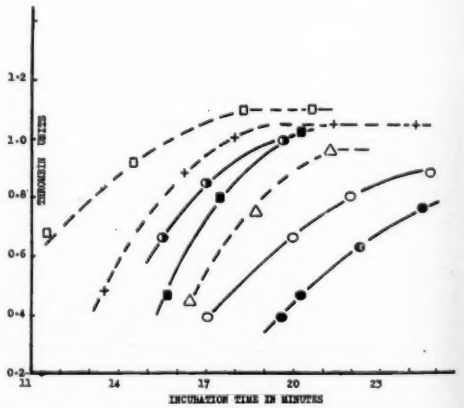

FIGURE XI

Influence of fresh citrated plasma on the rate of formation and yield of thrombin on blood partially deficient in α prothromboplastin. In each case 0·2 millilitre of 20% oxalated venous blood was used. (i) Patient's blood before transfusion (open circles). (ii) The same as (i) with the addition of 10% of donor's plasma (open rectangles). (iii) The same as (i) with the addition of 4% of donor's plasma (crosses). (iv) The same as (i) with the addition of 1% of donor's plasma (triangles). (v) Patient's blood eleven minutes after transfusion (half open circles). (vi) Patient's blood ninety minutes after transfusion (solid rectangles). (vii) Patient's blood forty-eight hours after transfusion (solid circles). The composition of the reaction mixture was the same as that given in Figure II (i).

serum prothrombin determinations were carried out sixty-five minutes after clotting in undiluted blood. These results are given in Table VII.

The results given in Table VII confirm the observation that the plasma had some corrective but very transient influence, as can be seen from the drop in whole blood clotting time at ten minutes and three hours after the transfusion. The serum prothrombin results are substantially in agreement with those obtained in Figure X.

We had the opportunity to examine a mild α prothromboplastin deficiency. Patient AG-1, aged forty-eight years, weight 56 kilograms, assumed blood volume four litres, was given intravenously 690 millilitres of citrated plasma, corresponding to 570 millilitres of native plasma, four hours after collection. The results of blood examinations prior to and after the transfusion are given in Figure XI and in Table VIII.

TABLE VIII

Serum Prothrombin Before and After Plasma Transfusion in α Prothromboplastin Deficient Blood

Time	Whole Blood Clotting Time; Two Millilitres (Minutes)	Serum Prothrombin 65 Minutes After Clotting		
		Quick (Seconds)	Prothrombin Assay (Units)	Percentage of Plasma Prothrombin
Before transfusion	25	24	86	15
Eleven minutes after transfusion	15	35	28	5
One and a half hours after transfusion	18	23	72	12
Forty-eight hours after transfusion	28	23	90	16

The results given in Figure XI indicate that the addition of approximately 4% of donor's plasma to the patient's blood *in vitro* was sufficient to produce a normal rate of thrombin formation (crosses). Yet 10% of donor's plasma introduced into the circulation produced an effect equal to about 4% α prothromboplastin activity (half open circles). The results given in Figure XI and Table VIII suggest that the hæmostatic effect of the plasma transfusion lasted for not much longer than ninety minutes.

Similar studies to those reported on the α prothromboplastin deficiencies were extended to β prothromboplastin deficiencies.

Influence of Normal Serum on the Rate of Formation and Yield of Thrombin In Vitro and In Vivo on Blood Devoid of β Prothromboplastin

Patient MA-2, aged thirty-eight years, weight 64 kilograms, with a blood volume assumed to be 4·5 litres, was given serum from normal donors twenty-four hours after clotting. The patient was given 500 millilitres of serum by vein over seventy minutes. Venous blood samples were taken prior to and after the transfusion, as indicated in Figure XII.

The results given in Figure XII show that *in vitro* the addition of between 2% and 5% of serum to the patient's blood produced a normal rate of formation and yield of thrombin (crosses, and solid circles). Blood specimens taken two and a quarter hours (open circles) and later after the conclusion of the transfusion gave results which indicated that approximately 1% of β prothromboplastin activity was present in shed blood, despite the fact that the patient obtained 10% of his blood volume as serum.

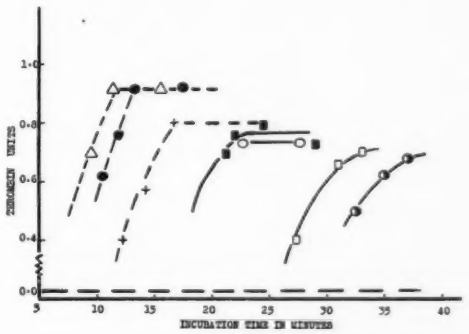

FIGURE XII

Influence of normal serum on the rate of formation and yield of thrombin *in vitro* and *in vivo* on blood devoid of β prothromboplastin. Patient MA-2; 0·2 millilitre of 20% oxalated venous blood was used in each case. (i) Patient's blood before transfusion (interrupted line). (ii) The same as (i) with the addition of 10% of donor's serum (triangles). (iii) The same as (i) with the addition of 5% of donor's serum (solid circles). (iv) The same as (i) with the addition of 2% of donor's serum (crosses). (v) Patient's blood two and a half hours after serum transfusion (open circles). (vi) The same twenty-four hours after transfusion (solid rectangles). (vii) The same forty-eight hours after transfusion (open rectangles). (viii) The same 120 hours after transfusion (half open circles). Other experimental conditions were similar to those given in Figure II (i).

Simultaneously with these investigations serum prothrombin determinations were carried out. These results are recorded in Table IX.

The results given in Table IX indicate that the transfusion of serum had some correcting influence two and a quarter hours after the transfusion. This can be seen from the improvement of the whole blood clotting time and from the prothrombin level in serum. However, the beneficial effect lasted less than forty-eight hours. The serum prothrombin results are substantially in agreement with those obtained in Figure XII. The results of the two independent techniques suggest that despite the fact that the volume of transfused

serum was approximately 10% of the blood volume, blood taken two and a quarter hours after transfusion appeared to have a considerably smaller β prothromboplastin activity than was expected. Apparently an unexpectedly large amount of the active serum factor disappeared from the circulation shortly after the transfusion. A possible explanation for the discrepancies between the in-vitro and in-vivo results is that serum contained the active β factor and plasma the precursor. Very often the active clotting factor is removed more quickly from the circulation than the precursor. Therefore, one hundred and twenty hours after the serum transfusion the patient was given intravenously 550 millilitres of citrated plasma

TABLE IX

Serum Prothrombin Before and After Serum Transfusion in Blood Devoid of β Prothromboplastin

Time	Whole Blood Clotting Time; Two Millilitres (Minutes)	Serum Prothrombin 65 Minutes After Clotting		
		Quick (Seconds)	Prothrombin Assay (Units)	Percentage of Plasma Prothrombin
Before transfusion	250	14	396	88
Two and a quarter hours after transfusion	17	19	95	21
Twenty-four hours after transfusion	32	28	60	10
Forty-eight hours after transfusion	27	17	230	45
One hundred and twenty hours after transfusion	45	15	380	60

five hours after collection, corresponding to 425 millilitres of native plasma. The results of blood examinations carried out after the plasma transfusion are given in Figure XIII.

It will be seen from the results given in Figure XIII that approximately 5% of donor's citrated plasma added to the patient's blood *in vitro* corrected the deficiency (solid circles). The transfused plasma volume was approximately 10% of the assumed blood volume. Three hours after the conclusion of the transfusion, which lasted forty-five minutes, blood activity corresponded to approximately 2% β prothromboplastin activity (open rectangles). Serum prothrombin determinations were also carried out (Table X).

The results given in Table X show that the transfusion of citrated plasma produced a drop in the whole blood clotting time to normal level, which perhaps lasted for eighteen hours; but the serum prothrombin figures indicate that the transfusion did not completely correct the deficiency. The plasma transfusion experiments are in agreement with the serum transfusion experiments, indicating that both twenty-four hours old serum and fresh citrated plasma are equally effective in correcting the clotting defect *in vitro*. Transfusion of either fluid in quantities greater than are considered adequate *in vitro* produced a considerably lesser effect when introduced into the circulation. This observation applied equally well to α prothromboplastin deficiencies as to β deficiencies just described.

A similar observation to those recorded in Figure XIII and Table X was made in another case of β prothromboplastin deficiency. In addition several other blood products were

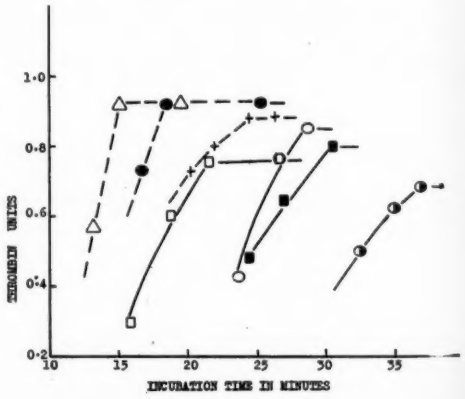

FIGURE XIII

Influence of citrated plasma on the rate of formation and yield of thrombin *in vitro* and *in vivo* on blood devoid of β prothromboplastin. Blood from patient MA-2 120 hours after serum transfusion. In each case 0·2 millilitre of 20% venous oxalated blood was used. (i) Before plasma transfusion (half open circles). (ii) The same as (i), with the addition of 10% of donor's plasma (triangles). (iii) The same as (i) with the addition of 5% of donor's plasma (solid circles). (iv) The same as (i) with the addition of 2% of donor's plasma (crosses). (v) Three hours after plasma transfusion (open rectangles). (vi) Eighteen hours after plasma transfusion (open circles). (vii) Forty-two hours after plasma transfusion (solid rectangles).

tested for their effect on the rate of thrombin formation *in vitro*. It was found that a dried plasma preparation which was prepared at least five years ago, reconstituted prior to use, was still very active *in vitro* as a source of the β factor. Human pooled serum filtered several times through a Seitz filter was inactive.

From the experience gained with the transfusion experiments, it appeared that in order to maintain satisfactory hæmostasis for longer periods in hæmorrhagic conditions due to prothromboplastin deficiencies, it might be necessary to resort to frequent intravenous administration of comparatively large volumes

of plasma or serum containing the missing factor. We had the opportunity to study the influence of plasma as well as serum given by drip infusion to a patient with complete β prothromboplastin deficiency who required operation for a hæmorrhagic cyst in the upper end of the right tibia.

TABLE X

Serum Prothrombin Before and After Transfusion of Citrated Plasma in Blood Devoid of β Prothromboplastin

Time	Whole Blood Clotting Time; Two Millilitres (Minutes)	Serum Prothrombin 65 Minutes After Clotting		
		Quick (Seconds)	Prothrombin Assay (Units)	Percentage of Plasma Prothrombin
Before transfusion	45	15	380	60
Three hours after transfusion ..	15	19	153	24
Eighteen hours after transfusion ..	17	14	198	30

Influence of Citrated Plasma or Serum Given as Drip Infusion on the Rate of Formation and Yield of Thrombin on β Prothromboplastin Deficient Blood

Patient MA-1, aged twelve years, 25·5 kilograms in weight, with an assumed blood volume of two litres, was given during a period of eleven days 3·5 litres of concentrated plasma, one litre of serum and 10 litres of citrated plasma. The results of blood examinations are given in Figure XIV.

The results given in Figure XIV show that the patient's blood prior to the transfusion did not produce any thrombin during an incubation time of fifty minutes (solid circles). The addition of 4% citrated plasma was just sufficient to produce thrombin normal in time and yield (open circles). For the drip infusion concentrated citrated plasma was used, which was obtained by freezing and removal of ice during thawing. It had a protein concentration of 10%, whereas the starting plasma contained 6% protein. It will be noted that the addition of 4% of this concentrated plasma corrected the deficiency *in vitro* (crosses). Two days after the start of the drip infusion, a blood examination indicated that β prothromboplastin activity was just within normal limits (triangles). A final examination was carried out eighteen hours after cessation of a ten days' continuous drip infusion, which indicated satisfactory hæmostasis (open rectangles).

CLINICAL MATERIAL

Detailed clinical observations are not recorded here as it is desired only to correlate clinical findings and laboratory data. Sufficient evidence is presented to indicate the nature and severity of the bleeding episodes. Except as it relates to the laboratory study, the genetics are not discussed.

Table XI gives the combined results of the clinical and laboratory data. Only six types of bleeding phenomena are listed, as these give the most useful information for comparative

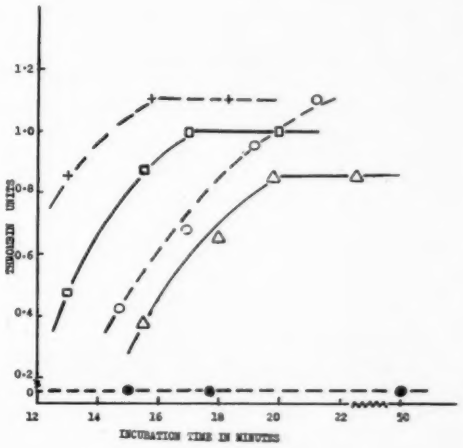

FIGURE XIV

Influence of citrated plasma or serum given as drip infusion on the rate of formation and yield of thrombin on β prothromboplastin deficient blood. In each case 0·2 millilitre of 20% oxalated venous blood was used. (i) Patient's blood before transfusion (solid circles). (ii) The same as (i) with the addition of 4% of concentrated plasma (crosses). (iii) The same as (i) with the addition of 4% of normal plasma (open circles). (iv) Patient's blood one day after operation, forty-eighth infusion (triangles). (v) Patient's blood ten days after continuous infusion (open rectangles).

purposes. There are, however, some features of the hæmophilic syndrome which are not tabulated, but which must be briefly mentioned. They are as follows.

Variability of Clinical Manifestations

No accurate grading of patients on the basis of clinical severity is possible, for the severity of the disease may be subject to wide fluctuation on both short and long term bases. Thus a patient who has had a severe clinical course during childhood may become relatively symptomless in adolescence, or one who has had a mild course in childhood may have his most severe bleeding during adolescence or even later in life. On a short-term basis, a patient who is experiencing severe bleeding episodes usually has periods of quiescence during which he can withstand considerable trauma without ill consequence. There is no general

TABLE XI

Clinical and Laboratory Data of 43 Male Bleeders Due to α Prothromboplastin or β Prothromboplastin Deficiency

Case Number	Patient	Age (Years)	First Hæmorrhage: Age (Years)	First Hæmorrhage: Cause	Acute Hæmarthrosis	Bruising	Dental Bleeding	Epistaxis	Intraabdominal Hæmorrhage	Hæmaturia	Blood Transfusions	Occupation	Laboratory Investigations: Whole Blood Clotting Time (Glass) in Minutes	Laboratory Investigations: Thrombin Formation: Units (Millilitres of Blood)	Laboratory Investigations: Thrombin Formation: Minutes	Laboratory Investigations: Serum Prothrombin in Percentage of Plasma Prothrombin
1	AA-1	8	8	Tooth extraction	−	−	+	−	−	−	0	——	15½	225	20½	10
2	AA-2	16	7	Epistaxis	−	±	+[1]	+	−	−	2	——	15½	191	20½	7
3	AA-3	63	25	Tooth extraction	−	−	+	−	−	−	0	Soldier, motor bus driver	15½	225	21	11
4	AB-1	31	2½	Laceration	−	−	+	+	−	−	0	Clerk	14–22	225	22	−
5	AC-1	45	8	Tooth extraction	+	+	+	±	−	−	0	Motor mechanic	16	220	23	−
6	AD-1	22	Neonatal	Circumcision	−	±	+	±	−	−	0	School teacher	16	216	25	17
7	AE-1	53	3	Laceration	+	++	+	±	−	−	0	Farm labourer	−	225	22½	−
8	AE-2	38	1	Laceration	+	++	+	+	−	−	3	Labourer	19	214	22½	−
9	AE-3	11	Neonatal	Circumcision	+++	++	+[1]	+	−	−	3	——	19	108	38	−
10	AP-1	8	Neonatal	Circumcision	+	±	0	±	−	−	1	——	25	207	23	28
11	AF-1	38	7	Laceration	+	++	+[1]	+	−	−	1	Shop proprietor	23	144	24	−
12	AF-2	20	10	Tooth extraction	−	−	+[1]	+	−	−	1	Shop assistant	16	225	24	−
13	AF-3	9	3	Laceration	−	−	+	−	−	−	−	——	−	144	25	−
14	AF-4	15	4	Laceration	++	±	+[1]	−	−	+	1	Shop assistant	20	158	28	−
15	AG-1	48	3	Laceration	−	+	+	+	−	−	1	Clerk	25	198	23	15
16	AG-2	36	Neonatal	Circumcision	±	++	+	+	−	−	0	Clerk	26	198	23	10
17	AH-1	15	Neonatal	Circumcision	++	+	+[1]	−	−	−	3	——	22	132	30	−
18	AH-2	20	Neonatal	Circumcision	++	+	0	±	−	−	−	Clerk	17½	156	27	−
19	AI-1	21	Neonatal	Circumcision	++	++	0	±	−	−	−	Farmer	19½	191	30	−
20	AJ-1	33	11	Tooth extraction	++	++	+[1]	±	−	−	3	Travelling salesman	23	171	28	30
21	AK-1	39	2	Laceration	++	++	+[1]	±	−	−	2	Motor truck driver	34½	158	42	−
22	AK-2	48	4	Laceration	++	++	+[1]	±	−	−	1	Soldier farmer	32	128	32	−
23	AL-1	16	10	Laceration	++	++	−	++	−	−	4	Gardener	29½	128	40	−
24	AM-1	26	1	Laceration	+	+	+[1]	++	−	−	1	Shop assistant	19½	159	45	−
25	AN-1	22	7	Tooth extraction[1]	++	−	+[1]	−	−	+	2	Motor truck driver	38	128	45	48
26	AO-1	24	2	Laceration	++	++	+[1]	−	−	−	4	Travelling salesman	32	164	32–48	37
27	GA-1	6	3 months	Hæmatoma	++++	++++	0	+[1]	−	−	>5	——	100	0	>60	−
28	GB-1	3	Neonatal	Circumcision	++	+++	+	−	+	+	>5	——	190	0	68	−
29	GC-1	9	6 months	Circumcision	+++	++	+[1]	+	−	+	>5	——	90	0	82	−
30	GD-1	7	9 months	Hæmatoma	+++	+++	+[1]	+	++	+	>5	——	162	0	>40	−
31	GE-1	21	3	Laceration	+++	+++	+[1]	+	+++	+	>5	Clerk	90	0	88	97
32	GF-1	22	1	Laceration	+++	+++	+[1]	±	−	+	>5	Tailor	100	0	88	76
33	GG-1	9	Neonatal	Circumcision	+++	++	+[1]	±	+	+	4	——	110	0	88	−
34	GH-1	21	Neonatal	Circumcision	+++	++	+[1]	±	−	++	4	Clerk	90	0	88	100
35	GI-1	16	3	Tooth extraction	+++	+++	+[1]	±	+	+++	>5	Invalid pensioner	70	0	88	90
36	GJ-1	13	Neonatal	Circumcision	++	+	+[1]	±	−	−	2	——	120	0	88	−
37	GK-1	6	Neonatal	Circumcision	+++	+++	+[1]	±	−	−	3	——	30	100	50–60	−
38	MA-1	12	6 months	Hæmatoma	+++	+++	+[1]	±	+	−	>5	——	120	0	88	−
39	MA-2	38	2	Laceration	+++	+++	+	++	++	+++	>5	Travelling salesman	240	0	88	86
40	MB-1	1¼	Neonatal	Circumcision	−	+++	0	−	−	−	>5	——	150	0	88	−
41	MC-1	11	1	Hæmatoma	++	++	+[1]	±	++	+	>5	——	240	0	88	−
42	MD-1	5	1	Hæmatoma	++	++	0	−	−	−	3	——	270	0	88	−
43	MD-2	4	Neonatal	Circumcision	+++	+++	0	−	+	+	3	——	240	0	88	75

[1]Blood transfusion given after dental extraction. ++++=very severe/very frequent/very numerous. +++=severe/frequent/numerous. ++=moderate/occasional/several. +=mild/rare/one or two. ±=slight or doubtful. −=not bleeding. 0=no teeth extraction.

tendency to improvement as a patient grows older, except as a direct result of taking greater precautions against injury. However, AN-1 (Case 25) used to suffer severe hæmarthroses during childhood, but now plays football with impunity. By contrast, AG-1 (Case 5) used to play football during his youth without injury, but now suffers from severe bruising after trivial trauma.

Variability in Severity of Clinical Manifestations in the Same Pedigree

In general the severity of symptoms suffered by patients of the same pedigree is similar. However, it is not identical. In the case of brothers, in which comparison is easy, the patients and parents sometimes state that one patient is more severely affected than the other. This is clearly seen in Cases 15 and 16 and in Cases 42 and 43.

However, it is true that in pedigrees of the milder forms of the disease the death of affected members occurs usually in late life and is not attributable to fatal hæmorrhage, whereas in the pedigrees of the more severe forms of the disease death usually results from hæmorrhage.

Heredity

When there is evidence of inheritance, all instances have been consistent with the sex-linked recessive mode. This applies to both α and β prothromboplastin deficiency diseases. It has been shown that in Cases 1 to 37 there is a deficiency of α prothromboplastin, and in Cases 38 to 43 a deficiency of β prothromboplastin. The latter group of cases have been reported by Fantl and Sawers (1954) as Cases I to VI.

Discussion

The presented investigations are concerned with defective thromboplastin formation in the shed blood of male patients. In the examination of hæmorrhagic disorders blood clotting time is often determined. In order to obtain reliable results, it can only be reiterated that a careful standardization of all procedures and apparatus is essential. However, it may be difficult to assess whether a blood specimen has been obtained free of tissue thromboplastin, the presence of which will shorten the reaction time and therefore invalidate the result. Simultaneous determination of the blood clotting time in glass and in silicone will be helpful, because the reaction of thromboplastin with other plasma components is not dependent upon the surface property of the container. In the case of a bad venous puncture, tissue thromboplastin may be introduced into the blood specimen in sufficient amount for the difference between clotting times in silicone and in glass to be small or absent. On the other hand, the conversion of thromboplastin precursors to thromboplastin is influenced by the surface property of the container. A properly collected blood specimen must therefore show a relatively big difference between the clotting times in glass and in silicone.[1] When all precautions are taken, the errors in the determination of blood clotting time are still very large. Therefore it is not advisable to rely on a single result. Experience over several years, which is condensed in this communication, has shown that repeated determinations of whole blood clotting time give comparable results for a given patient and are in agreement with the observations of Quick and Hussey (1952). This point is mentioned because some patients maintain that they experience periods of change in their bleeding tendency. In 37 patients it was noted with some surprise that the clotting times could be arranged in two clusters, with a wide gap between forty and seventy minutes. Biggs and Macfarlane (1953) made a similar observation in 50 cases of hæmophilia. In order to find an explanation for this phenomenon, mixtures of blood with a clotting time longer than seventy minutes and 2% normal blood were prepared. Such mixtures have given normal clotting times. This would indicate that the blood thromboplastin complex acts in an enzymatic manner, and not according to stoicheiometric laws, because in the latter case one would expect intermediate results. A delayed whole blood clotting time is some evidence of a disturbed coagulation mechanism, but a normal result does not exclude a clotting defect. Other techniques are necessary for the study of thromboplastin activity. In a qualitative manner α prothromboplastin deficiency (hæmophilia) can be established if the addition of fresh Ba-plasma to the patient's plasma shortens the plasma clotting time. Stored plasma is a satisfactory agent for the diagnosis of β prothromboplastin deficiency. But these two reagents form an incomplete thromboplastin system, and therefore do not give a quantitative result. Prothrombin remaining in serum after clotting has taken place is useful for the diagnosis of severe thromboplastin deficiencies, but the amount may be within normal limits in partial deficiencies. Further, it does not indicate the type or the degree of the deficiency. The

[1] This applies to coagulation deficiencies, but does not obtain in cases of recurrent thrombophlebitis due to carcinoma, in which often no difference between clotting times in glass and in silicone is observed, as we have seen in common with other workers (McCleery *et alii*, 1952).

determination of the rate and yield of thrombin produced from diluted blood in the presence of pyrocatechol has been used in all the reported cases as a quantitative measure of prothromboplastin. According to the results obtained from α prothromboplastin deficient blood, patients could be divided into three major groups. In the first no thrombin was formed. These patients are considered to be unable to produce α prothromboplastin. Blood specimens from the second group produced a fraction of the potential thrombin yield, although with much delay. These patients are considered to suffer from a moderate deficiency. Blood from the third group of patients produced the greatest possible thrombin yield, although somewhat delayed. These patients are considered to have a minor degree of prothromboplastin deficiency.

It is particularly important to take venous blood from β prothromboplastin deficient patients with great care, otherwise the plasma may clot in normal time. For this reason capillary blood, even in severe β prothromboplastin deficiencies, may behave normally. These observations indicate that β prothromboplastin deficient patients have a normal amount of tissue thromboplastin, and they suggest that α prothromboplastin is the precursor and the β factor is a co-factor essential for rapid conversion into plasma thromboplastin.

The prothromboplastin assay is a new technique, which has previously been used only in whole blood by Macfarlane and Biggs (1953), and in plasma by Pitney and Dacie (1953). It was therefore necessary to compare the results obtained with diluted blood with those from blood which has been interfered with as little as possible. A number of experiments with undiluted blood were carried out. The results are in substantial agreement with those obtained from diluted blood. Therefore the conclusions drawn from experiments with diluted blood are valid.

There are two schools of thought regarding the defect in bleeding tendencies connected with the thromboplastin complex. According to one, a deficiency is the cause of the defect—a view shared by a number of workers. According to the other school, an inhibitor masks thromboplastic activity. The latter view is maintained by Tocantins *et alii* (1951) and has recently been expressed by Johnson (1953). We can confirm Tocantins's observation that dilution of hæmophilic blood results in shortening of whole blood clotting time. Further, we noticed that undiluted fresh hæmophilic plasma, which did not produce any thrombin, became somewhat active even when stored at −15° C. The observations of many workers (for example, Fiehrer, 1950) that the euglobulin fraction obtained from hæmophilic plasma can produce thrombin on recalcification in a concentration not very much lower than that of normal plasma, is not surprising, in view of the fact that among the 43 patients investigated here only 11 were found to have a complete α prothromboplastin deficiency, and six a complete β deficiency. Twenty-six patients with α deficiency could produce from 50% to 100% of the potential thrombin. Further, it is not possible to draw conclusions from isolated plasma fractions because of the activation which takes place during the manipulations. A comparison of the rate of thrombin formation by blood partially deficient in α prothromboplastin with normal blood diluted to produce a similar reduction in thrombin yield has shown that normal blood produced thrombin still at a faster rate than the partially deficient blood. This suggests that a 50% yield in thrombin corresponds to a greater reduction than 50% in α prothromboplastin. This is further support for an enzymatic nature of thromboplastin activity.

The results obtained from mixtures of the two plasma thromboplastin precursors and thrombocytes indicated that normal blood has at least a fourfold excess of α prothromboplastin. From this it is concluded that bleeding tendencies of the α type can occur at any level between 0 and 25% of the normal. This is in agreement with findings of Graham *et alii* (1953). Thus it would appear that most of the evidence supports the assumption that the bleeding tendencies under discussion are due to deficiencies of essential clotting components. The different degrees of α prothromboplastin deficiencies are explained by the partial or complete absence or the partial or complete inactivity of a plasma factor required for blood thromboplastin activity. The first hypothesis assumes a material reduction of the essential factor, whereas the second assumes the production of a defective α prothromboplastin in the hæmophilic subject. The degree of the defect is controlled genetically. The most serious obstacle to accepting the theory of thromboplastin inhibitors as the cause of congenital hæmorrhagic disorders is that between 10% and 25% of normal blood is sufficient to correct the defect *in vitro*. One would have to postulate that normal blood contained a high concentration of substances with ability to neutralize the inhibitor. Further, bleeding tendencies due to the presence of inhibitors of the thromboplastin complex do exist, and it has been shown repeatedly that the addition of such blood specimens to normal

blood has always resulted in very considerable delay in clotting (Fantl and McLean, 1951). This is in contrast to what is found in deficiency conditions.

From the in-vitro experiments it was possible to establish the diagnosis as well as the degree of the deficiency. However, when a plasma or serum volume corresponding to that which corrected the defect *in vitro* was introduced into the circulation, the activity of either α or β prothromboplastin in blood specimens taken between ten minutes and two and a quarter hours after the transfusion was far lower than that calculated from a simple dilution of the particular factor in the assumed blood volume. Plasma thromboplastins are very probably complex proteins. It may be possible that they are metabolized with greater speed than the other blood proteins. However, this is not very likely. The other alternative is that α or β prothromboplastin after intravenous infusion is diluted into a space far greater than the blood volume. The obtained results suggest that this space is between two and three times greater than the blood volume.

With regard to the correlation between clinical symptoms and laboratory data, it would appear that there is some parallelism between the clinical severity of the hæmorrhagic condition and the degree of deficiency; but this is by no means absolute, and despite the fact that the coagulation defect was found to be constant in an individual patient and of identical degree in his affected male relatives, the clinical severity was often different and therefore at variance with the laboratory data. Furthermore, some patients showed at repeated examinations complete absence of α prothromboplastin in their blood, and yet considerable clinical improvement was noted. These observations suggest that hæmostasis is not only dependent upon the clotting mechanism, but requires in addition factors connected with vascular function, as has been noted previously (Fantl and Sawers, 1954).

Summary

Laboratory examinations on male bleeders—37 with α prothromboplastin deficiency (hæmophilia) and six with β prothromboplastin deficiency (PTC deficiency, Christmas disease)—are presented.

In addition to the more common tests, the coagulation properties were determined by the rate of formation and the amount of thrombin formed in diluted blood in the presence of pyrocatechol, which acts as a thrombin stabilizer.

The deficiencies of thromboplastin components could be corrected by the addition of blood or the appropriate blood products *in vitro*, and the degree of the deficiency of each patient's blood could be determined by the amounts required.

Variations from complete absence to minor deficiencies of α prothromboplastin were observed, whereas only complete absence of β prothromboplastin was encountered.

Normally the rate of formation of thrombin is faster in capillary blood than in venous blood, owing to contamination by tissue thromboplastin in the former. However, in cases of complete α prothromboplastin deficiency, venous blood and capillary blood give identical results. On the other hand, in cases of complete β prothromboplastin deficiency, only properly collected specimens of venous blood give reliable results, whereas capillary blood may produce erroneously short thrombin clotting times. Apparently patients with complete β prothromboplastin deficiency have enough tissue thromboplastin to mask the results in capillary blood. These observations suggest that the labile α prothromboplastin may be the precursor of the blood thromboplastin complex, and the β factor an essential co-factor for rapid thromboplastin formation.

Experiments have been presented which indicate the catalytic nature of the blood thromboplastin complex. Normal blood has at least a fourfold excess of the thromboplastin components. A bleeding tendency is therefore expected to occur below a level of 25% of the normal plasma concentration.

Whole blood clotting time and the rate of thrombin formation were constant in any one patient; an identical degree of deficiency was present in the blood of affected male relatives.

In-vivo studies show that for satisfactory hæmostasis, far greater amounts of the missing factor have to be given intravenously than is suggested from the in-vitro results. This would indicate that the rate of destruction of the plasma thromboplastin component is greater than that of other blood proteins, or that the transfused α prothromboplastin or β prothromboplastin occupies a space two to three times the blood volume.

A comparison between the laboratory data and clinical findings shows that in general there is some parallelism between the clinical severity of the hæmorrhagic tendency in a pedigree, and the degree of blood deficiency. However, this is by no means absolute. It was noticed that in contrast to the constancy of laboratory findings, the clinical manifestations varied greatly. This, of course, would indicate that in addition to the blood deficiency, other factors, presumably of vascular nature, play an important part in the clinical manifestations.

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QUANTITATIVE ASPECTS OF FILTER PAPER ELECTROPHORESIS OF SERUM[1,2]

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THE proteins of serum may be resolved into characteristic electrophoretic components by passing an electric current across a buffer-soaked strip of filter paper to which serum has been applied. Following this separation, the protein fractions may be demonstrated by staining the paper strip with certain dyes (Cremer and Tiselius, 1950; Durrum, 1950). Measurement of the amount of dye bound to these components permits an electrophoretic pattern to be constructed, which agrees very closely in form with a Tiselius type pattern of the same serum. Filter paper electrophoresis employs the same principles as Tiselius electrophoresis, but requires neither elaborate nor costly apparatus, and is well suited to the clinical laboratory investigation of serum protein abnormalities.

Many variables influence protein movement in a paper electrophoretic system; however, under standard conditions in alkaline buffers, the final location of a given protein on a paper strip is mainly determined by the following: (*a*) the electrophoretic migration of the protein towards the anode, (*b*) the electro-endosmotic flow of buffer towards the cathode, and (*c*) the flow of buffer towards the centre of the strip from the two ends of the paper under the influence of evaporation (Macheboeuf *et alii*, 1953). Evaporation effects are minimized by suspending the paper strip in a closed water-vapour saturated chamber (Grassmann and Hannig, 1952; Durrum, 1951; Flynn and de Mayo, 1951), or abolished either by immersion of the strip in a chlorbenzol bath (Cremer and Tiselius, 1950; Schneider, 1951), or by clamping it between siliconed glass plates (Kunkel and Tiselius, 1951). This report is concerned with the electrophoretic separation of serum protein fractions on paper in a vapour-saturated box, the subsequent measurement of the individual protein components, and their alterations in diseased states.

METHODS

The types of apparatus described by Grassmann and Hannig (1952) and by Durrum (1951) were employed. In both, the filter paper strip (Whatman Number 1) was freely suspended

FIGURE I

The Durrum type of electrophoretic apparatus. There are two inner buffer compartments, 34 by 7·0 centimetres, into which the paper dips, and two outer electrode compartments, 34 by 2·0 centimetres. The paper strips are supported 20° from the vertical by a plastic rack of apex height 18·5 centimetres

in a vapour-saturated chamber, hanging horizontally with tension in the former apparatus, and semi-vertically over an inverted V-shaped rack in the latter (Figure I). Serum (0·01 millilitre) was applied with a micropipette to the buffer-damped paper strip as a band. A diethylbarbituric acid-sodium barbital buffer, pH 8·6, ionic strength 0·1, was used. Electrophoresis was carried out for sixteen hours at 4° C., after which the papers were dried and

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stained for ten minutes with amido black 10B (Grassmann and Hannig, 1952)[1]; the protein-free paper was decolorized by washing with 10% acetic acid in methanol. The serum protein components appeared as separate dye-stained bands, the relative amounts of which were measured by an optical photometer.[2] The paper was passed over a one millimetre light slit, and the light absorption was registered by a photocell in optical density units. When the optical densities (ordinate) were plotted against millimetre distance along the paper strip (abscissa), an electrophoretic pattern resulted. The planimeter area beneath each peak was proportional to the amount of protein component present, the percentage of each being expressed as percentage of total protein, representing total planimeter area. Other details of method have been reported elsewhere (Mackay *et alii*, 1954). This photometric procedure is considerably quicker than elution techniques, which involve cutting the strips into small segments, from which the dye is eluted and measured colorimetrically (Cremer and Tiselius, 1950; Flynn and de Mayo, 1951; Kunkel and Tiselius, 1951).

Serum lipids were stained with a saturated solution of Sudan black (Swahn, 1954) for twelve hours. Amyl alcohol appeared superior to ethyl alcohol as a stain solvent. The Sudan-stained paper was decolorized with water washes, although considerable background stain remained.

[1] Good results were also obtained with azocarmine dye (Turba and Enenkel, 1950; Mackay, Volwiler and Goldsworthy, 1954).

[2] Obtained from Bender and Hobein, Munich, Germany (Grassmann and Hannig, 1952).

RESULTS

The reproducibility of paper electrophoretic analysis of serum protein by this method is illustrated by multiple (eight) analyses on a single abnormal serum, and by comparison of duplicate analyses of eleven different sera, chiefly abnormal (Tables I and II). These

TABLE I

Results of Multiple Analyses of a Single Abnormal Serum Specimen (Tuberculous Adenitis)[1]

Observation	Albumin	Globulins			
		α_1	α_2	β	γ
Means (eight analyses)	37·3	5·6	13·4	11·4	32·3
Maximum differences ..	3·5	1·3	2·4	2·2	4·7
Maximum differences from mean	1·8	0·8	1·5	1·2	2·7
Standard deviations from mean	1·20	0·46	0·77	0·61	1·72

[1] Serum components expressed as percentage of total protein.

and other data suggest that the method is quantitatively reliable, although poor resolution of certain abnormal sera can lead to large differences between duplicates. For example, resolution of β from γ globulin may be poor in portal cirrhosis, and duplicate differences of up to 10% of total protein occasionally occurred in measuring the γ globulin fraction in cirrhosis. In most cases, however, multiple analyses of serum should give values agreeing within 3% of total protein for albumin and γ globulin, and 2% for the other fractions.

Results obtained by photometric analysis of stained paper strips were compared with free electrophoretic analyses in two series of patients (Table III). For these sera, the values of each

TABLE IIa

Duplicate Analyses of Eleven Normal and Abnormal Sera[1]

Diagnosis	Determination I					Determination II				
	Albumin	Globulins				Albumin	Globulins			
		α_1	α_2	β	γ		α_1	α_2	β	γ
Normal	54·6	4·3	8·9	11·6	20·6	54·6	3·4	9·1	11·3	21·6
Normal	60·7	2·2	7·8	13·7	15·6	63·4		7·3	12·7	16·5
Portal cirrhosis ..	44·6	2·7	6·4	10·8	35·5	40·0	4·5	6·9	10·0	38·5
Biliary cirrhosis ..	37·8	13·4		19·5	29·3	36·0	13·6		18·9	31·5
Myeloma	36·8	4·2	8·0	9·6	41·4	39·3	5·2	6·8	9·4	39·3
Myeloma	24·7	3·4		65·1	6·8	27·8	4·8		62·8	4·6
Essential hypercholesterolæmic xanthomatosis.. ..	60·4	2·7	7·4	14·3	15·1	56·9	2·6	8·3	15·3	16·9
Lupus erythematosus	26·7	3·1	8·3	11·0	50·9	24·0	3·5	6·5	10·5	55·5
Lupus erythematosus	40·0	7 4	14 1	11·7	24 8	42·4	6·3	13·3	10·5	27·5
Myeloid leuchæmia	45·1	8·2	11·9	9·0	25·8	45·7	7·3	13·7	9·4	23·9
Pulmonary abscess ..	40·4	5·5	11·6	14·2	28·3	40·6	4·9	12·2	15·0	27·3

[1] Serum components expressed as percentages of total protein.

component obtained by free and paper electrophoresis agreed well, and the differences were mainly within the range of experimental variation; for the series as a whole, β globulin values were somewhat lower and γ globulin values higher by paper than by free electrophoresis. With certain abnormal sera the

TABLE IIB

Observation	Albumin	Globulins			
		α_1	α_2	β	γ
Maximum difference between duplicates ..	4·6	1·8	1·8	2·3	4·6
Means of differences between duplicates ..	2·2	0·9	0·8	0·8	2·1
Standard deviations between duplicates ..	2·58	1·03	1·02	1·00	2·36

results differed more widely. Sources of divergence in results between the two methods may be explained as follows:

1. The photometer readings were proportional to the amounts of protein on the paper strip only within certain limits of protein concentration. Known amounts of albumin in 0·01 millilitre of solution were applied as a band to paper strips and stained with amido black. The optical density values across the band were plotted, and the areas of the resulting graph were determined by planimetry. The relationship of areas to amounts of protein was linear up to albumin concentrations of 1·6 grammes *per centum*, beyond which photometer measurements of albumin were falsely low. This factor would account for discrepancies observed with narrow dense bands of myeloma protein (Table IV, Cases 34 and 35).

2. Migration of albumin along the paper strip is accompanied by adsorption of protein to the paper. Such adsorption or "trailing" was shown to account for reductions of 10% of the albumin value (Table IV, Case 6).[1]

3. The photometric measurement of dye bound to equal amounts of serum albumin and γ globulin suggests that serum albumin has an intrinsically greater dye-binding capacity than homogeneous γ globulin by a factor of 1·3.

4. The great difference in α and β globulin percentages between paper and free electrophoretic analyses of sera of high lipid content suggests that lipid components of lipoprotein complexes are not stained by protein-binding dyes, although such components do contribute to refractive change in free electrophoresis (Table IV, Cases 28 and 40).

Electrophoresis of serum lipids and lipoproteins was found to be technically unsatisfactory in our hands. Lipid bands were poorly delineated, probably owing to adsorption of lipid on the paper. Moreover, it is apparent that with our technique Sudan black stains some serum protein as well as serum lipids. This subject has been extensively investigated by Swahn (1954).

The following summarizes observations upon alterations of serum proteins in various clinical syndromes as revealed by paper electrophoresis:

1. Normal subjects. The mean percentage values[2] (12 subjects) for all components were as follows: albumin 57·4, α_1 globulin 4·2, α_2 globulin 8·5, β globulin 14·0, and γ globulin 15·9.

2. Advanced portal cirrhosis. The albumin was low (mean 34·9%), α and β globulins

[1] The protein "trailing" error appeared to be increased with buffers of low ionic strength.

[2] Expressed as percentages of total serum protein as represented by total planimeter area.

TABLE III

Comparison of Paper and Free Electrophoretic Values[1]

Clinical Condition	Paper Electrophoresis					Free Electrophoresis				
	Albumin	Globulins				Albumin	Globulins			
		α_1	α_2	β	γ		α_1	α_2	β	γ
Normal:										
Means—12 subjects	57·4	4·2	8·5	14·0	15·9	57·4	5·0	8·5	15·2	13·9
Mean differences from free electrophoresis ..	0	−0·8	0	−1·2	+2·0	—	—	—	—	—
Portal cirrhosis:										
Means—13 subjects	34·9	5·2	8·7	12·8	38·4	31·9	4·7	8·0	14·1	41·3
Mean differences from free electrophoresis (7 subjects)	+0·9	+0·9	+0·3	−3·0	+0·9	—	—	—	—	—

[1] Serum components expressed as percentages of total protein.

variable, γ globulin increased (mean 38·4%). The γ band was broad and diffuse, and a characteristic finding was the poor resolution of the β and γ bands. This was not believed to be a technical difficulty, as this merging together of the β and γ areas occurred also with our methods of free electrophoresis (Figure IIc). In the more severe and terminal cases in which there was pronounced elevation of γ globulin, a separate and distinct band appeared between the β and γ areas, representing the γ_1 component (Figure II), which amounted to 10% to 12% of the total serum protein. This component was occasionally detected in lower concentrations in other conditions producing hypergammaglobulinæmia.

3. Advanced biliary cirrhosis. The albumin was low and the β globulin high; the β globulin component was not as strikingly shown by paper as by free electrophoresis. In certain cases the γ globulin values were also elevated, and in one case a γ_1 band appeared.

4. Essential hypercholesterolæmic xanthomatosis. The β globulin was increased, the serum albumin was normal.

TABLE IV

Cases Illustrating Application of Paper Electrophoresis[1]

Diagnosis and Case Number	Electrophoretic Analysis	Total Protein (Grammes *per Centum*)	Albumin	Globulins					Remarks
				α_1	α_2	β	γ_1	γ_2	
Normal (6) —	Paper	7·3	60·3	4·5	8·9	14·0	—	12·3	
	Paper (with low ionic strength buffer)	—	49·3	5·3	13·3	16·5	—	11·9	10% reduction in albumin concentration due to "trailing"
	Free	—	59·2	4·9	8·9	15·1	—	15·6	
Portal cirrhosis (25)	Paper	5·8	32·4	11·5	10·5	10·4	12·3	22·9	γ_1 component demonstrable by paper electrophoresis only
	Free	—	33·0	9·1	13·1	10·5	—	34·3	
Portal cirrhosis (71)	Paper	8·4	29·9	4·5	5·2	14·8	—	45·6	Inaccurate measure of globulins due to poor resolution between β and γ bands on paper strip
	Free	—	26·9	3·2	5·2	10·5	—	54·2	
Biliary cirrhosis (28)	Paper	6·7	33·7	7·3	11·6	18·2	5·6	23·6	Markedly lower β globulin values by paper electrophoresis in sera of high lipoprotein content
	Free	—	26·4	6·3	9·7	29·9	—	27·7	
Essential hypercholesterolæmic xanthomatosis (40)	Paper	7·65	60·4	2·7	7·4	14·3	—	15·1	
	Free	—	46·0	5·5	10·2	27·7	—	10·6	
Multiple myeloma (34)	Paper	11·4	24·7	3·4	—	65·1	—	6·8	Dense myeloma protein band not accurately measured by photometer
	Free	—	20·0	2·4	—	75·1	—	2·5	
Multiple myeloma (35)	Paper	9·2	28·4	5·8	8·6	7·0	—	50·2	
	Free	—	20·1	5·0	7·0	6·7	—	61·2	
Glomerulonephritis (89)	Paper	3·4	24·6	5·4	58·7	—	—	11·3	"Lipoid nephrosis" with giant α_2 peak obscuring β component. Ultracentrifugation of serum to remove lipid caused no change in protein components.
	Paper[2]	—	24·5	5·3	60·1	—	—	10·1	
	Free[2]	—	21·7	6·4	60·1	—	—	11·7	
Osteomyelitis and empyema (96)	Paper	6·4	29·4	6·3	12·8	11·9	7·2	32·4	Note α_2 elevation and γ_1 component
Lupus erythematosus (42)	Paper (before cortisone)	8·7	24·0	3·5	6·5	10·5	—	55·6	Marked reduction in γ globulin following cortisone therapy
	Paper (after cortisone)	7·9	29·6	7·5	11·3	12·3	—	39·3	
Agammaglobulinæmia (55)	Paper	5·9	64·4	4·4	10·4	17·8	—	3·0	Three-year-old male, recurrent skin infections
	Free	—	66·3	5·5	11·5	14·7	—	2·0	
Agammaglobulinæmia (69)	Paper	6·2	57·3	5·9	15·7	14·6	—	6·5	Fifteen - year - old male, chronic empyema
	Free	—	57·1	8·3	15·1	15·1	—	4·4	

[1] Serum components expressed as percentage of total protein.

[2] Lipæmic serum cleared by Spinco ultracentrifugation, 40,000 revolutions per minute, for twelve hours, preparatory to free electrophoresis.

5. Multiple myeloma. In the twelve cases observed, the usual finding was a dense discrete band in one of the globulin areas, most often occupying the γ position; in cases in which there was a considerable increase in β globulin, very little protein was present in the usual γ area (Figure V). This dense sharply outlined protein band occurred in no other conditions, and contrasted strikingly with the diffuse γ globulin band of cirrhosis and chronic inflammatory conditions; it thus had diagnostic value. One normoproteinæmic patient with myeloma was encountered, but no case of the rare α globulin variety.

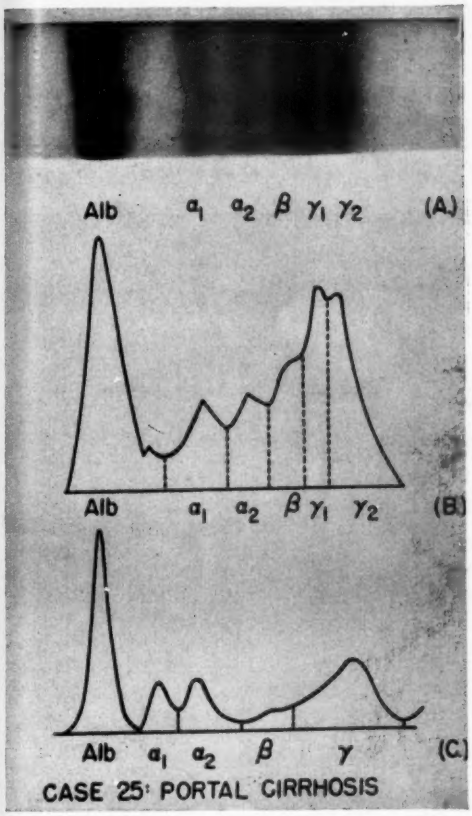
Alb α_1 α_2 β γ_1 γ_2 (A)
Alb α_1 α_2 β γ_1 γ_2 (B)
Alb α_1 α_2 β γ (C)
CASE 25: PORTAL CIRRHOSIS

FIGURE II

Comparison of dyed paper electrophoretic strip (A) and corresponding photometer pattern (B) with Tiselius schlieren diagram (C). Case 25: Portal cirrhosis (terminal) with increased α_1 globulin.

6. The nephrotic syndrome. This was characterized by very low albumin values, slightly elevated α_1 concentration, greatly elevated α_2 and β globulin concentrations, and a variable γ globulin level. The eleven patients with this condition included some with glomerulonephritis, diabetic nephropathy, and toxæmia of pregnancy. All these conditions leading to this syndrome seem to have produced a common type of "nephrotic" pattern (Figures III and V).

7. Acute and chronic inflammatory conditions of a septic nature. In these there was a slight reduction of albumin, a definite rise in α_2 globulins, and occasionally some increase in γ globulin (Table IV and Figure V, Case 96).

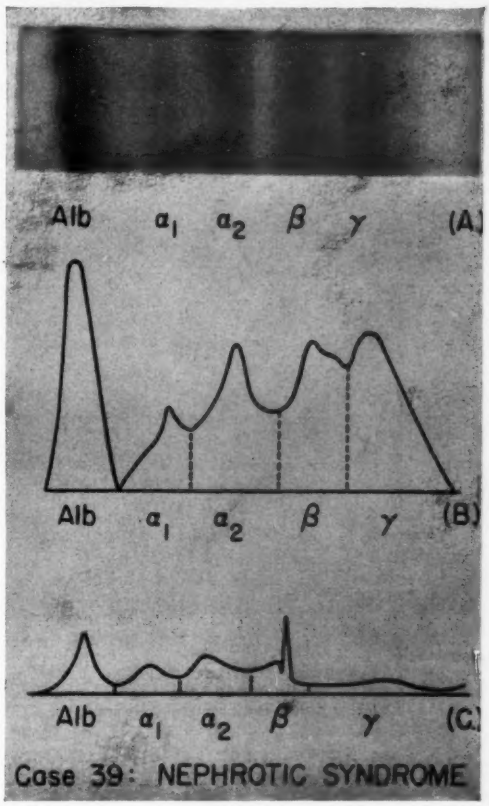

FIGURE III

Comparison of dyed paper electrophoretic strip (A) and corresponding photometer pattern (B) with Tiselius schlieren diagram (C). Case 39: Toxæmia of pregnancy, nephrotic syndrome; low albumin and increased α_2 and β globulins

8. Miscellaneous, non-septic chronic inflammatory conditions (rheumatoid arthritis, *lupus*

erythematosus, collagen diseases *et cetera*). In these there was a considerable increase in γ globulin, sometimes with the appearance of a definite γ_1 component. Paper electrophoretic analyses of the serum of a patient with *lupus erythematosus* revealed a pronounced reduction in γ globulin concentration after cortisone therapy (Table IV, Case 42).

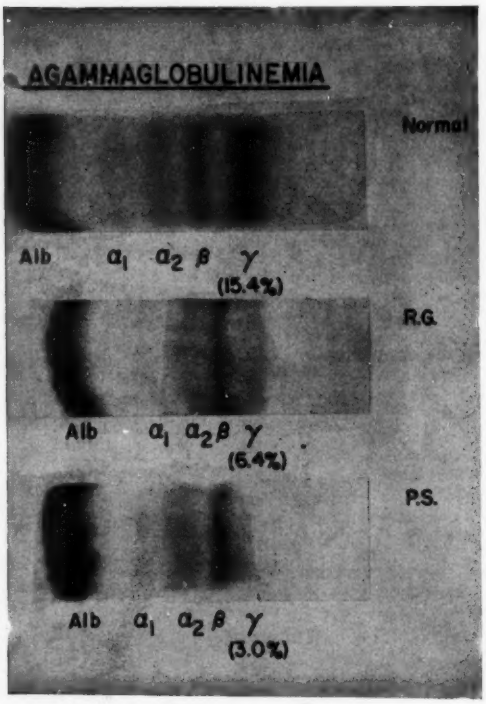

FIGURE IV

Agammaglobulinæmia. The serum of R.G. (Case 68) shows a faint α globulin band; with the serum of P.S. (Case 55) there is no α globulin band

9. Agammaglobulinæmia. Four examples of this syndrome have been recognized in the past year, including one lesser degree of the condition in which very reduced amounts of γ globulin were present in the serum (Table IV, Cases 55 and 68, and Figure IV).

DISCUSSION

Quantitative reproducibility of paper electrophoretic analysis of serum appears satisfactory and agrees with other published data (Grassmann and Hannig, 1952; Flynn and de Mayo, 1951; Sommerfelt, 1952). Precision of analysis is not claimed to equal that of free electrophoresis. It has been emphasized (Sommerfelt, 1952) that expressing differences between duplicates in terms of total protein may be misleading; for example, a duplicate difference in smaller fractions, such as α_1 globulin, of even 1% of total protein can represent at least a 20% error in measuring actual concentration.

The relationship of results obtained by paper electrophoresis to those from free electrophoretic analysis has been the subject of controversy. Using bromphenol blue stain, followed by elution and colorimetry, earlier investigators reported that, to achieve comparability with free electrophoresis, optical

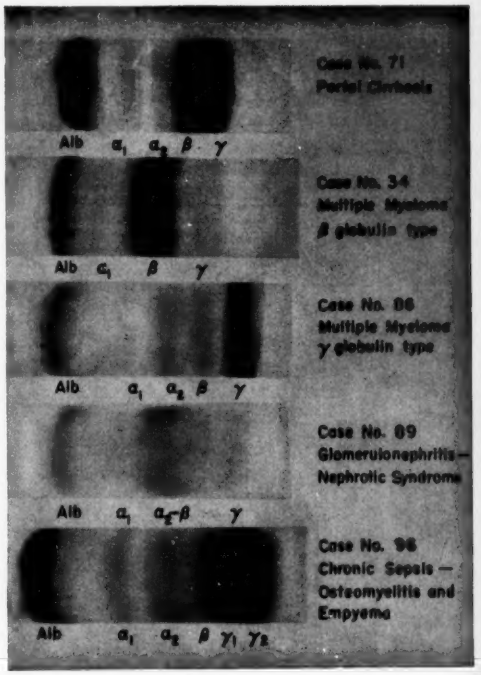

FIGURE V

Characteristic paper electrophoretic patterns of serum in various abnormal conditions

density values over the whole globulin range should be multiplied by a certain factor; some authors (Cremer and Tiselius, 1950; Flynn and de Mayo, 1951; Schneider, 1951; Goa, 1951) used a constant factor such as 1·6, whereas Köiw *et alii* (1952) found this globulin factor to be quite variable. However, when we have used direct photometric quantitation, it has been our experience (Mackay *et alii*, 1954) as well as that of Sommerfelt (1953) and of

Griffiths (1953), that no such factor is required. It is uncertain whether these dye-elution and photometric methods of quantitating the separated fractions give varying results; analysis by both methods of three separate serum samples revealed no significant differences (Mackay *et alii*, 1954).

Four possible sources of difference between paper and free electrophoretic analysis have been investigated. In normal and most abnormal sera, these discrepancies produce a minimal total effect in so far as paper and free electrophoretic comparison is concerned; slightly lower β globulin values, and slightly higher γ globulin values are obtained by the paper technique. With certain abnormal sera, containing very high concentrations of myeloma protein or having increased lipoprotein content, analyses by the two methods are much less consistent.

The diagnostic applications of this technique are limited by the non-specificity of the electrophoretic pattern of abnormal sera, and there are few situations in which a diagnosis cannot be arrived at more conclusively by simpler means. Electrophoretic analysis is of diagnostic importance in the following situations:

1. In agammaglobulinæmia the diagnosis can best be established by electrophoretic procedures. Cases may be suspected in children with severe recurrent infections, cutaneous pulmonary, or any type, which are poorly resisted. Diagnosis is important, as good therapeutic responses have been reported after administration of γ globulin.

2. Some cases of multiple myeloma may be detected electrophoretically before the marrow aspirate findings become diagnostic.

3. Progress and response to therapy in conditions such as portal cirrhosis, the nephrotic syndrome, the "collagen diseases" *et cetera*, may be followed by serial electrophoretic analyses of serum.

SUMMARY

Studies of serum protein resolution by paper electrophoresis, and photometric quantitation of the dye-stained paper strip are described. Direct photometric measurement was found to be quick and reproducible. Quantitative differences existing between paper and free electrophoretic analyses are attributable to various factors inherent in the paper technique; these differences are significant only in certain abnormal sera. Protein abnormalities in various disease states are described. Filter paper electrophoresis demonstrates well characteristic electrophoretic changes in conditions of serum protein derangement, but precise diagnostic information is obtained in few situations—that is to say, in agammaglobulinæmia, in multiple myeloma, and in following the progress of diseases associated with abnormal serum proteins.

ACKNOWLEDGEMENT

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FILTER PAPER ELECTROPHORESIS OF SERUM PROTEINS IN CLINICAL PRACTICE[1]

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The importance of the study of the plasma and serum proteins under various pathological and physiological conditions has long been acknowledged, but until quite recently it has been dependent mainly on two techniques, salt fractionation and electrophoresis by the moving boundary method of Tiselius. Both methods yield fractions of a roughly similar nature, since separation depends essentially on the properties of charge, shape and size of proteins. Each suffers from definite disadvantages. Salt fractionation, especially if carried beyond the determination of a simple albumin-globulin ratio, is time-consuming and not always reliable. Moving boundary electrophoresis requires expensive equipment and places severe limitations on the number of specimens that can be dealt with at one time. Nevertheless, by means of these methods (especially the latter) extensive knowledge regarding the changes undergone by the serum and plasma proteins in health and disease has been gathered. This has been reviewed by Gutman (1948).

The recent introduction of filter-paper electrophoresis for separation of the proteins of serum or plasma (Wieland and Fischer, 1948; Turba and Enenkel, 1950; Cremer and Tiselius, 1950; Durrum, 1950) has made available a method which has most of the advantages and few of the disadvantages of previous methods.

We have now used it for over two years, and in 1953 it was adopted as the routine technique for the estimation of serum proteins at the Brisbane General Hospital, and the salt fractionation technique was abandoned.

Since the introduction of the Tiselius moving boundary method, the desire has often been expressed for a pattern which might be attached to the patient's chart as we have electrocardiograms. With the advent of the new method this is now possible. However, while a great deal may be learnt from a qualitative inspection of the pattern, especially by a practised observer, we thought that it would be more helpful if such a qualitative appraisal could be reinforced by a simple and reasonably reliable quantitative technique. This communication deals with the application of filter-paper electrophoresis in clinical practice and presents some of the results we have obtained by its means.

Method

The method depends on the fact that different groups of proteins have different speeds of migration in an electric field.

We have used essentially the technique of Durrum (1950), as modified by Flynn and de Mayo (1951).

A small quantity of serum is applied to a sheet of filter paper, which is saturated with barbitone buffer of pH 8·6. A current is then passed at 150 volts across the paper. We used initially Whatman Number 3 filter-paper, but when this became unavailable changed to Whatman Number 1, which has proved satisfactory. After three to five hours the paper is removed, dried and stained to show the position and concentration of the protein fractions. The stain used is bromphenol blue, which is taken up by the protein from acid solution and is released in alkaline solution, the protein acting as a sort of ion exchanger. This fact is used in quantitative estimation, the stained pattern being cut into its separate fractions which are placed in a fixed volume of 0·01 N caustic soda solution, and the eluted colour is read in a colorimeter.

Pooled normal human serum obtained from the Red Cross blood bank has been used as a standard of reference, and this is run with each specimen. The normal values assigned to the pooled serum are based on those of Franklin *et alii* (1951). The results from the unknown are assessed by the ratio of the colorimeter reading for a given fraction of the unknown to that of a similar fraction of the "standard".

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This is converted to grammes *per centum* by means of a calibration curve.

It has become customary in the electrophoresis of serum and plasma to employ a buffer of pH 8·6. This appears to yield the greatest number of components. The electrophoretic pattern for normal (pooled) serum at pH 8·6 is shown in Figure I. The fractions were named as follows by Tiselius (starting with the fraction with greatest anodal mobility): albumin, and α_1, α_2, β and γ globulins. The γ globulin can at times be seen to have two components subsequently called γ_1 and γ_2.

Plasma shows a further band between the β and γ globulins—namely, that of fibrinogen.

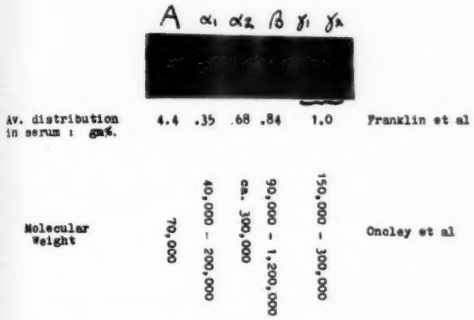

FIGURE I

The electrophoretic pattern for normal (pooled) serum

Figure I also shows the average distribution of serum protein between the fractions together with their molecular weight.

The copper sulphate method as described by Phillips *et alii* (1945) was used for estimation of total serum protein.

RESULTS

The data are presented in Tables I to V. They are derived from routine serum protein investigations carried out at the Brisbane General Hospital. We have given special attention to collagen disease, the nephrotic syndrome, hepatic cirrhosis and multiple myeloma, for it is in these diseases that the electrophoretic changes are the most characteristic and of greatest value in diagnosis or assessment of the patient's condition.

DISCUSSION

Collagen Disease

Figure II (*b*) shows the fully developed pattern of collagen disease. It is from a young girl with active rheumatoid arthritis. There is depression in the albumin and increase in the α_2 and γ globulins. The β band is split. We have come to call this the typical "collagen disease pattern".

Table I shows the values obtained for the various serum protein fractions in 28 cases of collagen disease. Blood sedimentation rates are shown also for comparison. It will be seen that in only one case was the γ globulin less than one gramme *per centum*, and this was complicated by the co-existence of exfoliative dermatitis. The albumin level was invariably decreased and the α_2 globulin raised. We found the β globulin band to be frequently split, and this has been commented on before by Wallis

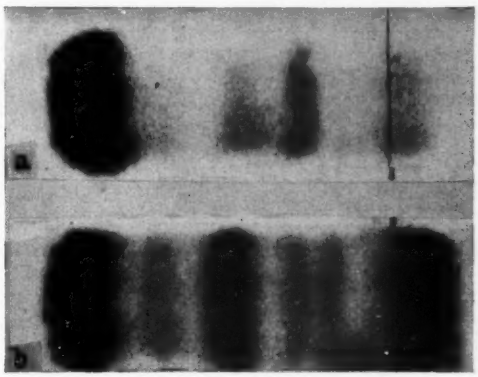

FIGURE II

Collagen disease; (*a*) standard (pooled) serum; (*b*) fully developed pattern of collagen disease (active rheumatoid arthritis)

(1950). The "β_2" component may disappear just by keeping the specimen in the refrigerator. In any case it is not a specific change; we have seen it in a case of abdominal abscess. Neither are the changes in the other fractions in themselves specific.

A prompt fall in the serum albumin level is a common feature of any inflammatory process, injury, burn *et cetera*. The exact nature of this is still not clear.

The association of α_2 globulin with "inflammation or tissue destruction irrespective of its causes" (Shedlovsky and Scudder, 1942) was recognized by the earliest investigators and has been confirmed many times since. As such it is an excellent indicator of the extent to which these processes occur in the organism.

Gamma globulin is practically always increased after infection of more than a fortnight's duration, the level subsiding again

once the infection has been overcome. A long-continued and persistent rise occurs in collagen disease, in chronic infections, in most of the reticuloses and in hepatic cirrhosis.

The γ globulin actually consists of two elements, γ_1 and γ_2. The γ globulin of normal serum is predominantly of the γ_2 type. In infection and collagen disease the γ globulin increase is confined to components with a mobility of γ_2 globulin.

Table II (*a*) illustrates the electrophoretic changes in the serum of a boy, aged fifteen years, with recurrent rheumatic fever. The disease was at its height at the time of the first two sets of values, and in remission at the time of the third, and recurred shortly before the last. The blood sedimentation rates are also shown.

Table II (*b*) similarly illustrates the series of changes in a man, aged thirty-nine years, with severe rheumatic fever treated with cortisone. The first set of values shows the results before treatment with cortisone, the succeeding ones after cortisone given at the rate of 300 milligrammes on the first day, 200 milligrammes on the second day and thereafter 100 milligrammes per day.

TABLE I

Collagen Diseases

Patient or Subject	Age (Yrs.)	Sex	Total Protein (Grammes *per Centum*)		Albumin (Grammes *per Centum*)	Globulin Fractions (Grammes *per Centum*)				Blood Sedimentation Rate (Millimetres in One Hour)	Associated Conditions
			Copper Sulphate	Addition		α_1	α_2	β	γ		
Normal subject	—	—	—	—	4·4	0·35	0·68	0·81	1·00	—	
					Rheumatic Fever						
M.H.	33	F.	6·7	7·3	2·9	0·58	1·5	1·0	1·30	32	
W.E.	38	M.	7·5	8·0	2·1	0·50	1·9	0·98	2·50	30	
J.P.	14	M.	6·8	6·4	3·0	0·40	0·98	0·65	1·40	35	
B.N.	14	F.	7·7	7·4	2·9	0·48	1·20	0·75	2·05	32	
J.F.	8	M.	—	5·9	2·5	0·65	0·88	0·90	1·05	(Pre-cortisone, 22)	
L.W.	12	M.	6·8	—	3·8	—	0·90	0·95	1·40	12	
C.C.	12	F.	7·8	7·6	4·0	0·48	1·02	0·77	1·30	10	
L.F.	28	F.	7·5	7·4	3·6	0·50	1·12	0·85	1·30	34	
C.B.	39	M.	6·7	7·0	2·8	0·46	1·18	0·96	1·60	30	
C.H.	32	M.	—	7·6	3·8	0·42	0·98	1·02	1·42	19	
					Rheumatoid Arthritis						
H.S.[1]	66	M.	5·7	5·8	2·8	0·54	0·80	0·65	1·00	31	Lung abscess
C.E.	16	F.	7·4	7·1	3·4	0·52	1·05	0·80	1·30	20	
G.M.	13	M.	8·3	8·4	4·2	0·50	0·77	1·15	1·80	25	
M.E.[2]	55	M.	—	5·5	2·7	0·48	0·93	0·62	0·75	—	Exfoliative dermatitis
A.V.	60	M.	5·9	6·0	2·3	0·57	1·25	0·70	1·20	30	
W.K.	36	F.	6·8	6·5	2·9	0·46	0·96	0·66	1·55	32	
W.B.	41	M.	6·8	7·2	2·6	0·43	1·35	1·03	1·80	32	
F.W.	30	M.	6·0	6·0	2·5	0·36	1·10	0·75	1·33	35	
V.T.	16	F.	7·9	6·9	2·6	0·40	1·20	1·20	1·50	30	
G.G.	63	M.	8·2	8·3	3·9	0·45	1·35	0·95	1·65	—	
J.M.	19	F.	6·8	7·5	2·8	0·41	1·20	0·70	2·40	33	*Lupus erythematosus*
V.T.	20	F.	5·2	4·8	1·5	0·27	1·50	0·65	1·25	40+	*Lupus erythematosus* (with nephrotic syndrome)
E.M.	29	F.	7·0	7·0	3·3	0·46	1·03	0·92	1·29	17	*Lupus erythematosus*
J.S.	24	F.	6·8	7·1	3·3	0·49	1·10	0·86	1·30	30	*Erythema nodosum*
M.M.	19	F.	7·1	7·1	3·6	0·42	1·02	0·90	1·15	—	*Erythema nodosum*
L.G.	35	M.	6·2	6·2	2·8	0·47	0·97	0·80	1·20	—	*Polyarteritis nodosa*
I.N.	61	M.	7·3	7·5	3·6	0·35	1·05	0·75	1·75	22	Scleroderma
W.P.	14	M.	7·0	7·1	3·9	0·35	0·77	0·82	1·30	12	Scleroderma
Mean ..	—	—	6·87	6·91	3·07	0·46	1·11	0·85	1·46	—	
Standard deviation of mean ..	—	—	1·34	0·82	0·88	0·08	0·42	0·15	0·12	—	

[1] Lung abscess also present.
[2] Exfoliative dermatitis also present.

TABLE II

Progressive Estimations in Two Cases of Rheumatic Fever

Patient and Date	Albumin (Grammes *per Centum*)	Globulin Fractions (Grammes *per Centum*)				Blood Sedimentation Rate (Millimetres in One Hour)
		α_1	α_2	β	γ	
(*a*) J.P.						
12/8/52	2·3	0·73	1·24	0·72	1·05	30
7/9/52	2·1	0·68	1·45	0·71	1·57	35
30/1/53	3·4	0·42	0·80	0·80	1·10	12
9/7/53	3·0	0·40	0·98	0·65	1·40	35
(*b*) C.B.						
26/6/53	2·8	0·46	1·18	0·96	1·60	30
30/6/53	2·8	0·44	1·02	1·08	1·40	18
3/7/53	3·1	0·46	0·85	1·12	1·38	7
14/8/53	3·9	0·35	0·82	1·14	1·14	6
Normal	4·4	0·35	0·68	0·84	1·00	—

Nephrotic Syndrome

Figure III (*b*) shows the typical pattern associated with the nephrotic syndrome. The albumin is decreased to very low values, the α_1 globulins are normal or slightly depressed, the γ globulins are considerably lowered, while the α_2 globulins are much increased. The β globulins are slightly increased or within normal limits.

Table III demonstrates the values obtained in 22 cases of the nephrotic syndrome. Included in the list is a case due to *lupus erythematosus* and one due to amyloidosis.

The mean for the albumin concentration was 1·83 grammes *per centum*. The mean for the α_2 globulin concentration was 1·31 grammes *per centum*, the highest value being 2·30 grammes *per centum*. The mean figure for the γ globulins was lowered to 0·70 gramme *per centum*, the lowest value being 0·38 gramme *per centum*.

Figure III (*d*) shows another example of the nephrotic syndrome, and Figure III (*e*) the corresponding urinary pattern. All the serum protein constituents are represented in the latter.

TABLE III

Nephrotic Syndrome

Patient	Age (Years)	Sex	Total Protein (Grammes *per Centum*)		Albumin (Grammes *per Centum*)	Globulin Fractions (Grammes *per Centum*)				Remarks
			Copper Sulphate	Addition		α_1	α_2	β	γ	
C.P.	15	F.	6·3	6·9	3·4	0·32	1·40	0·83	0·95	
B.S.	16	M.	3·4	4·7	1·9	0·32	1·15	0·75	0·60	
D.J.	40	M.	4·0	4·4	1·4	0·35	0·95	0·84	0·80	
J.M.	41	M.	3·7	5·2	1·3	0·40	2·20	0·75	0·52	
B.S.	3	M.	3·0	4·4	1·4	0·27	1·10	0·90	0·70	
C.B.	53	M.	3·2	4·4	1·5	0·31	1·20	1·00	0·40	
B.L.	26	F.	3·3	3·6	1·2	0·32	0·61	0·74	0·70	
G.T.	2	M.	3·5	4·0	0·8	0·30	1·80	0·70	0·38	
G.H.	14/12	M.	2·8	3·7	0·5	0·22	1·30	1·05	0·58	
K.W.	23	M.	7·1	7·5	3·8	0·42	1·20	1·00	1·05	Proteinuria only
L.T.	4	F.	3·2	3·7	0·7	0·30	1·35	0·67	0·75	
J.G.	63	M.	4·1	4·7	2·1	0·29	0·90	0·80	0·63	
G.P.	5	F.	—	6·6	2·3	0·41	2·30	1·05	0·57	
L.H.	66	F.	4·0	3·6	0·55	0·28	1·35	0·70	0·75	
M.B.	19	F.	4·4	5·6	2·3	0·36	0·98	0·96	0·95	
L.B.	2	F.	6·1	7·1	3·3	0·60	1·58	1·06	0·52	
W.A.	60	M.	6·4	6·8	3;7	0·41	1·02	0·80	0·88	
J.B.	32	F.	4·7	5·4	2·8	0·38	0·85	0·75	0·60	Hypertension, gout
P.S.	64	M.	3·7	4·2	1·4	0·28	1·02	0·76	0·78	? Following mersalyl
E.J.	79	F.	4·3	4·7	1·5	0·29	1·18	1·08	0·62	? Following mersalyl
V.T.	20	F.	4·6	5·0	1·1	0·23	2·00	0·60	1·10	*Lupus erythematosus*
D.K.	27	M.	4·6	4·6	1·2	0·33	1·50	0·88	0·66	Amyloidosis
Mean ..	—	—	4·31	5·03	1·83	0·34	1·31	0·85	0·70	
Standard deviation of mean ..	—	—	1·17	1·17	0·98	0·08	0·30	0·14	0·18	

Table III shows a systematic difference between values for total protein obtained by the copper sulphate method and by addition of separate fractions. Several factors may account for this. The large quantity of protein-bound

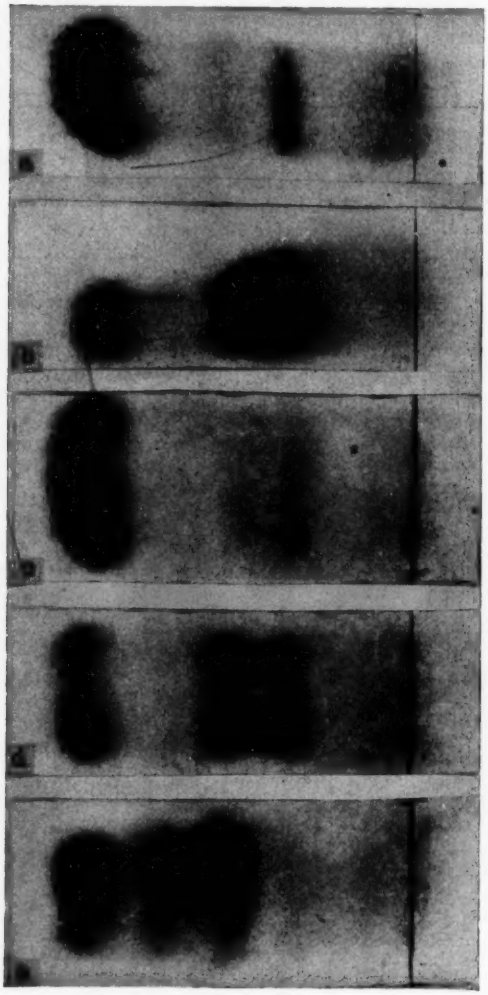

FIGURE III

Nephrotic syndrome; (*a*) standard; (*b*) typical nephrotic pattern; (*c*) standard; (*d*) nephrotic pattern with (*e*) urinary protein pattern from the same case.

carbohydrate associated with the α_2 globulins would take up bromophenol blue, and also the very high lipid of the β globulin would probably depress the specific gravity measured by the copper sulphate method. The latter also accounts for the large β peak seen in electrophoretic patterns obtained by the moving boundary technique, in which material registers through the change of refraction it causes in the medium.

Hepatic Cirrhosis

Hepatic cirrhosis is accompanied by a decrease in all fractions except the γ globulins, which

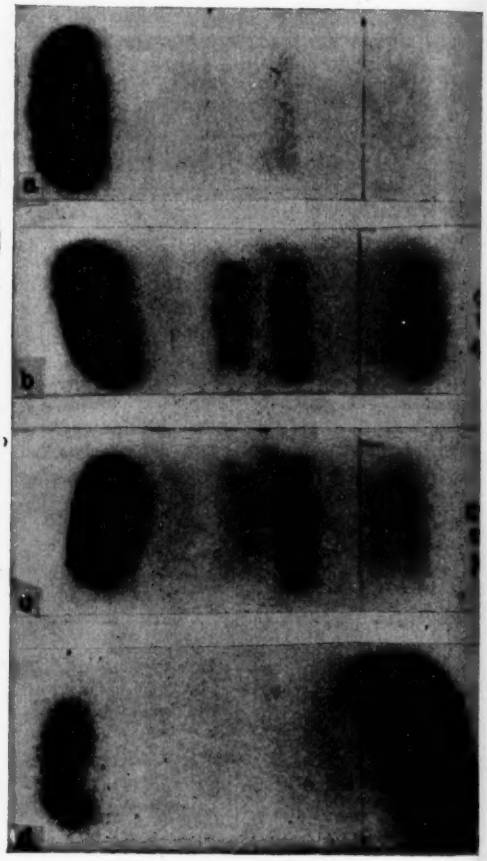

FIGURE IV

Liver diseases; (*a*) standard; (*b*) pattern from case of severe infective hepatitis (G.C., Case I; see Table V, May 26, 1953); (*c*) standard; (*d*) typical cirrhotic pattern

are increased. Figure IV (*b*) shows the typical electrophoretic pattern of advanced cirrhosis, and Table IV the values obtained in 22 cases.

The mean for the γ globulin was 1·80 grammes *per centum*, as against the normal of 1·0 gramme *per centum*; the highest value was 2·90 grammes *per centum*.

TABLE IV

Cirrhosis

Patient	Age (Yrs.)	Sex	Total Protein (Grammes *per Centum*)		Albumin (Grammes *per Centum*)	Globulins (Grammes *per Centum*)				
			Copper Sulphate	Addition		α_1	α_2	β	Total γ	γ_1[1]
G.O.	70	M.	7·5	7·2	3·2	0·48	0·71	0·70	2·05	0·45
M.M.	63	F.	6·3	5·8	2·4	0·28	0·35	0·68	2·10	0·50
C.L.	48	M.	7·2	7·2	3·8	0·34	0·62	0·90	1·50	+++
J.C.	60	M.	5·6	5·5	3·0	0·33	0·40	0·60	1·20	Nil
A.I.	70	M.	6·6	6·7	3·8	0·46	0·51	0·80	1·15	+
C.P.	33	M.	6·1	5·7	2·7	0·32	0·51	0·70	1·50	–
E.G.	56	M.	6·6	6·1	2·4	0·56	0·92	0·75	1·45	–
E.F.	59	F.	6·6	6·0	2·6	0·29	0·58	0·75	1·75	0·40
H.L.	50	M.	7·5	6·9	2·3	0·27	0·52	0·95	2·83	0·43
P.C.	33	M.	8·0	8·2	4·4	0·35	0·62	1·00	1·85	0·35
G.G.	8	F.	7·3	7·7	3·7	0·37	0·82	0·75	2·10	–
E.L.	63	M.	7·1	7·1	3·8	0·33	0·57	0·95	1·40	++
R.G.	73	M.	7·0	6·6	3·1	0·43	0·87	0·90	1·30	+
G.I.	50	M.	6·6	6·5	2·7	0·40	0·51	0·80	2·08	+++
I.G.	61	F.	7·6	7·5	3·7	0·38	0·75	0·80	1·90	–
A.H.	54	M.	6·8	7·2	4·2	0·37	0·54	0·60	1·50	–
O.L.	55	F.	6·5	6·7	3·6	0·35	0·61	0·86	1·26	++
C.K.	61	M.	4·7	5·1	2·4	0·30	0·57	0·70	1·12	–
F.McG.	44	M.	5·7	5·7	1·7	0·25	0·48	0·55	2·70	0·35
M.R.	76	F.	5·3	6·0	1·4	0·38	0·68	0·67	2·89	0·54
R.B.	41	F.	7·1	7·8	3·5	0·40	0·52	0·80	2·55	0·30
J.K.	51	M.	7·3	7·3	3·8	0·38	0·75	0·92	1·40	–
Mean ..	—	—	6·39	6·42	3·10	0·35	0·61	0·78	1·80	—
Standard deviation of mean ..	—	—	1·50	1·97	0·78	0·07	0·15	0·13	0·54	—

[1] γ_1: " + ", 0·25 to 0·35 (approximately); " ++ ", 0·35 to 0·45; " +++ ", 0·45 or more; " – ", not determined separately.

Although the rise in total γ globulin in cirrhosis was noted early during the application of the electrophoretic method, the specific meaning of an increase in the γ_1 globulins was not universally recognized until the work of Franklin *et alii* (1951). We have consistently confirmed the association of a rise in the γ_1 globulins with cirrhotic liver. It is an especially valuable aid in cases in which liver damage is suspected although the albumin level is maintained near normal. In only 15 of our cases was γ_1 globulin separately estimated. In 11 of these the concentration was found to be twice normal or more (normal, 0·18 gramme *per centum*).

Other Hepatic Disease

The presentation of tables for infectious hepatitis is somewhat meaningless unless these are correlated exactly with the stage of the disease, and the following clinical histories are intended to illustrate the changes in the serum protein fractions in two representative cases.

CASE I.—G.C., a man, aged sixty-two years, was suffering from infective hepatitis of considerable severity (subacute hepatic necrosis). He was admitted to hospital on April 7, 1953. He had a history of duodenal ulcer and also of nephrolithiasis leading to nephrectomy fourteen years earlier. He had been in his usual health until eleven days before his admission to hospital, when he had several rigors and felt nauseated. On the following day he vomited. Nausea persisted and he vomited with increasing frequency. Jaundice was observed nine days after the commencement of his illness. On his admission to hospital he was deeply jaundiced and had a very much enlarged liver. His spleen was also moderately enlarged. Vomiting was so intractable that it was necessary to feed him intravenously during the second week. By April 18, however, he had greatly improved, and though he remained very ill for another month he made steady progress thereafter and was discharged from hospital on June 23, 1953. However, his liver remained considerably enlarged.

Biochemical investigations gave the following results. The serum bilirubin level two days after his admission to hospital (April 9) was 11·2 milligrammes per 100 millilitres; by May 8 it was 0·8 milligramme per 100 millilitres. The cephalin cholesterol flocculation test produced a positive result throughout; on April 9 the result was " +++ " in twenty-four hours and " +++ " in forty-eight hours; on June 22 it was still " ++ " in twenty-four hours, " +++ " in forty-eight hours. On April 9 the serum alkaline phosphatase level was 17 King-Armstrong units per 100 millilitres.

Numerical values for electrophoretic fractions are shown in Table V (*a*) and the pattern on June 19 in Figure IV (*b*).

CASE II.—This was an average case of infective hepatitis. The patient, D.T., a female, aged thirty years, was admitted to hospital on May 27, 1953. She had been well until ten days earlier, when she became anorexic and feverish. Two days later she observed her urine to be dark in colour, and three days later again she noticed that she was jaundiced. On her admission to hospital the serum bilirubin content was 6·3 milligrammes per 100 millilitres. On Jule 1 the cephalin cholesterol flocculation test result was positive (twenty-four hours " ++ ", forty-eight hours " ++++ "). The serum alkaline phosphatase content was 11·3 King-Armstrong units

per 100 millilitres. Her progress thereafter was steadily towards recovery. The jaundice disappeared after two weeks, during which time her appetite had returned. By July 20 the only evidence of liver dysfunction was in the biochemical data, the cephalin cholesterol flocculation test result still being strongly positive (twenty-four hours " +++ ", forty-eight hours " +++ "). A week later, however (July 27) the result was much more weakly positive (twenty-four hours " + ", forty-eight hours " + "), and on August 4 she was discharged from hospital.

The numerical values for electrophoretic fractions are shown in Table V (*b*).

When the moving boundary method was first applied to clinical material, it was hoped that it might furnish a means of differentiating decisively obstructive jaundice from other types of hepatic disease. In the former a large peak appeared between the α_2 and β globulins, which could be removed by extracting the serum with ether. On the other hand, in infectious hepatitis and cirrhosis large elevations of the γ globulins were found.

TABLE V

Progressive Estimations in Two Cases of Infective Hepatitis

Patient and Date	Albumin (Grammes *per Centum*)	Globulins (Grammes *per Centum*)			
		α_1	α_2	β	γ
(*a*) G.C.					
12/4/53	3·3	0·35	0·58	0·65	1·30
25/4/53	2·7	0·32	0·85	0·70	1·35
9/5/53	3·4	0·42	0·80	0·80	1·30
26/5/53	3·4	0·40	0·65	0·70	1·60
5/6/53	3·5	0·32	0·63	0·72	1·50
19/6/53	3·9	0·43	0·82	0·90	1·60
(*b*) D.T.					
29/5/53	3·6	0·35	0·57	0·85	1·60
10/6/53	3·7	0·28	0·61	0·95	1·40
24/6/53	3·6	0·37	0·65	0·72	1·30
20/7/53	3·9	0·35	0·64	0·94	1·10

The large β peak has been subsequently identified as the abnormal lipoprotein of obstructive jaundice. Additional abnormalities are inversion of the cholesterol-phospholipid ratio from approximately 1·4 to 0·5, and absence of α lipoprotein (Kunkel and Slater, 1952). However, lipid is not detected by the bromophenol blue staining technique, although at times there is evidence of a band between the α_2 and β globulins. The lipid changes in biliary obstruction can be demonstrated in filter paper electrophoresis by special staining techniques (Swahn, 1952), and we have observed the absence of the α lipoprotein band in a number of cases.

Unfortunately the divisions are not so clear-cut as expected. In the early stages of infectious hepatitis many of the features of obstruction may be present because actual obstruction exists owing to swelling of the parenchymal cells.

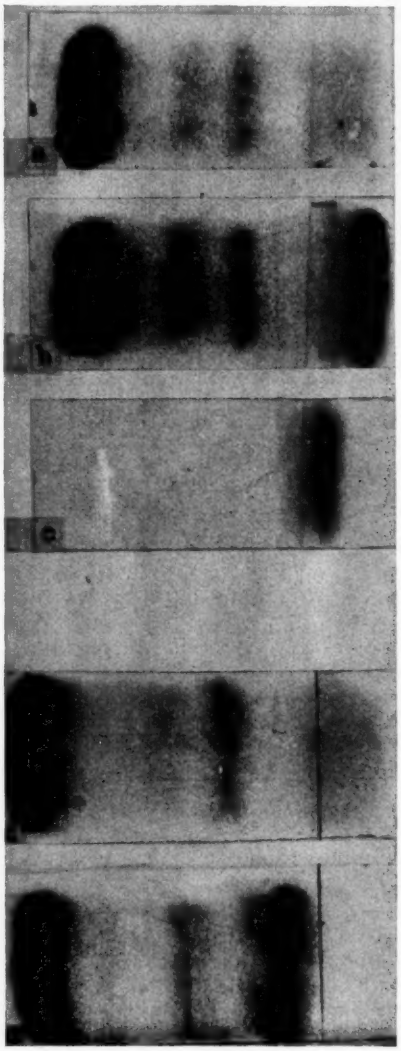

FIGURE V

Multiple myeloma ; (*a*) standard ; (*b*) multiple myeloma with very slow moving band ; (*c*) urinary protein from same case (see text) ; (*d*) standard ; (*e*) multiple myeloma with band having mobility slightly less than β globulin

On the other hand, a raised γ globulin level may exist in obstructive jaundice, owing to the presence of infection other than infectious hepatitis.

The relation of other liver function tests to electrophoresis has been dealt with by Gutman (1948). It may be briefly summarized by stating that the cephalin cholesterol flocculation reaction depends on quantitative changes in the γ globulin and on both quantitative and qualitative changes in the albumin. The Takata-Ara reaction is simply a rough test of the amount of γ globulin. Similarly the zinc sulphate turbidity test result correlates well with total γ globulin levels; the thymol turbidity, on the other hand, is also affected by β lipoprotein. The colloidal gold and similar reactions are more complex, but depend essentially on the interactions of albumin, γ globulin and β lipoprotein.

We have found the cephalin cholesterol reaction the only one which was likely to give information not supplied by electrophoretic methods.

Multiple Myeloma

The main and most striking abnormality in the pattern of multiple myeloma is a sharply localized, compact band of abnormal γ globulin, travelling with a mobility between that of the β globulin and that of the slowest of the γ globulins. Since the quantity of protein in the band is large and the homogeneity great, it tends to spread laterally. Figure V (*e*) shows a multiple myeloma band of mobility slightly less than that of β globulin. Figure V (*b*) illustrates a case in which the mobility is less than that of normal γ globulin, and Figure V (*c*) shows the electrophoretic pattern of urine from the same patient demonstrating Bence-Jones protein. The mobility of this is greater than that of the abnormal serum component. (However, no correction was made for pH, and it is possible that this is responsible for the apparent difference in mobility — Brueckner, 1954, personal communication).

In the group of 15 cases of multiple myeloma examined, the distribution of mobilities was as follows: in four the mobility was intermediate between β and γ, in six the mobility was that of ordinary "γ" globulin (that is, γ_2) or even slower, and in two the mobility was that of β globulin. In three cases no characteristic abnormality was seen.

The abnormal proteins ranged in amount from 1·5 to 5·0 grammes *per centum*

SUMMARY

The use of filter-paper electrophoresis with a simple method of quantitative estimation as a routine diagnostic investigation is described, the significance of the fractions obtained is discussed and the results in certain diseases are presented. In a number of cases the pattern is fairly specific and can be of great use in diagnosis; these are as follows: (i) Collagen disease: albumin depressed; α_1 and α_2 globulins much increased; β globulin low, normal or split; γ globulin increased. (ii) Nephrosis: albumin very low; α_1 globulin subnormal; α_2 globulin greatly elevated; β globulin variable; γ globulin diminished. (iii) Cirrhosis: albumin low; α_1, α_2 and β globulins decreased or variable; γ_1 and γ_2 globulins much increased. (iv) Multiple myeloma: sharply localized band of abnormally homogeneous γ globulin with a mobility of anything between that of β and that of very slow γ globulin.

The method has been found of value in assessing the progress and activity of a disease process, especially in collagen disease or infectious hepatitis. It can replace many previous methods, such as most of the flocculation or turbidity tests used in the latter. It can also act as a valuable adjunct to others, such as estimation of the blood sedimentation rate in collagen disease.

The importance of serial determinations is stressed, and clinical histories are presented in which such determinations were made in untreated collagen disease, in collagen disease treated with cortisone, and in infectious hepatitis.

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AN EVALUATION OF LIVER FUNCTION TESTS INCLUDING FILTER PAPER ELECTROPHORESIS[1]

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A WIDE variety of liver function tests has been introduced for the purpose of determining the presence and nature of liver disease. These tests yield much valuable information, but their very multiplicity tends to create confusion. There appears to be a tendency to increase the number of tests performed rather than to obtain the maximum information from those available in a routine laboratory.

In view of this tendency we carried out a series of standard liver function tests and in most cases a liver biopsy on 170 patients who had various liver diseases. Only those tests which could be performed in a clinical laboratory were used. Analysis of serum proteins by means of paper electrophoresis was carried out in addition, so that estimates of serum albumin and serum globulin concentrations could be obtained. Correlations have been made, not only between the results of liver function tests and the patient's disease, but also between the values of the various tests themselves.

The purpose of this paper is to point out that a few standard liver function tests are adequate for diagnosis and for assessing the progress of patients with liver disease. Emphasis is placed on the tests which have been found most useful and their particular applications are discussed.

METHODS

The methods used were as follows.

The serum bilirubin concentration was estimated by the method of Malloy and Evelyn (1937). The upper limit of normal was regarded as 1·0 milligramme per 100 millilitres.

The serum alkaline phosphatase concentration was determined by the method of King and Armstrong (1934). The upper limit of normal was regarded as 13 units.

The prothrombin index, although estimated in most cases, is not discussed throughout the paper, as a large number of patients were given injections of vitamin *K* before being referred to the hospital, hence no true evaluation of this test can be made.

The total serum protein concentration was estimated by the falling-drop method (Phillips *et alii*, 1944). The normal range was taken to be between 6·0 and 8·0 grammes *per centum*.

The thymol turbidity test was carried out by Shank and Hoagland's (1946) modification of Maclagan's (1944) method. The zinc sulphate turbidity was determined by the method of Kunkel (1947). The optical density in both these tests was measured in a photometer and the results were expressed as the O.D. × 100. The upper limit of the normal range in this laboratory for both thymol turbidity and zinc sulphate turbidity as obtained from 100 blood bank donor samples was 15 units.

The bromsulphthalein retention was carried out by the method of Neefe and Reinhold (1946). Five milligrammes per kilogram of bromsulphthalein were injected intravenously and the retention of dye was determined by a photoelectric colorimeter from a specimen of blood taken forty-five minutes later. The upper limit of normal retention was regarded as 6%.

The serum albumin and γ globulin concentrations were estimated by paper electrophoresis, with the use of a " Veronal " buffer of pH 8·6. The dried filter paper was stained with bromphenol blue, washed and dried again. Fractions were eluted from the paper with sodium carbonate-methanol, and the dye concentration was estimated in a Beckman spectrophotometer. The upper limit of normal of γ globulin was regarded as 1·5 grammes *per centum*.

All zinc sulphate turbidity and thymol turbidity tests, estimations of bromsulphthalein retention and liver biopsies were carried out by one of us (D.C.) and all the electrophoretic estimations by the other (C.R.B.B.).

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The remaining tests were performed by the staff of the Biochemistry Department of the Fairfax Institute of Pathology and by the staff of the Clinical Research Unit Laboratory.

MATERIAL

Blood was taken from eight patients who had no clinical or histological evidence of liver disease, and from 170 patients with liver disease. The latter were grouped as shown in Table I.

TABLE I

Diseases Studied

Condition	Number of Cases	Number of Biopsies
Acute infective hepatitis	44	7
Continuing hepatitis	30	28
Cirrhosis	41	34
Extrahepatic biliary obstruction ..	15	15
Secondary neoplasm of the liver ..	11	11
Hæmochromatosis	5	5
Miscellaneous liver disease	24	21
Total	170	121

RESULTS

The data presented and expressed graphically in this section are those obtained on the initial investigation of the patients. Serial tests were carried out on most patients and the results of these will be discussed in a later section of the paper.

The data will be presented firstly in terms of the disease from which the patients were suffering, and secondly in terms of the individual tests and their correlations.

THE DISEASES

No Apparent Liver Disease

Eight patients in whom there was no clinical evidence of liver disease had liver biopsies taken either by needle or at laparotomy. Both the results of liver function tests and the histological appearances were within normal limits in every case.

Acute Infective Hepatitis

A clinical diagnosis of acute infective hepatitis was made in 44 cases, but only seven of the patients had a liver biopsy and histological confirmation of the diagnosis. Most of these patients were observed during an epidemic in a neighbouring suburb and no patient is included in this series whose clinical diagnosis was in doubt. Nine patients had no jaundice on presentation, but all except two had undoubted evidence of jaundice during the course of their disease.

The data from these patients are shown graphically in Figure I.

The zinc sulphate turbidity was increased in 43 of these 44 cases, whereas the thymol turbidity was normal in seven. The bromsulphthalein retention was estimated in too few cases for the data to be of any significance. The serum alkaline phosphatase concentration was normal in 15 out of the 41 cases in which it was determined, in 22 the levels were those usually accepted as diagnostic of acute hepatitis (less than 35 King-Armstrong units), but in four levels greater than 35 King-Armstrong units were found. These last four

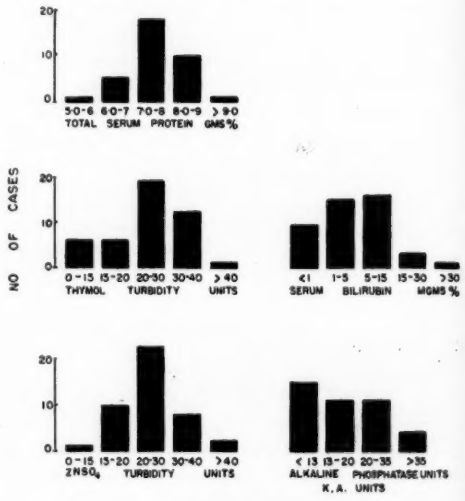

FIGURE I

The distribution of the results of five liver function tests in 44 cases of acute infective hepatitis

patients had protracted courses before completely recovering. The serum bilirubin concentration was within the expected range of from one to 15 milligrammes per 100 millilitres in 31 cases; in one the level was 40 milligrammes per 100 millilitres and the patient had a protracted course but completely recovered. The serum γ globulin concentration was determined in 11 cases, in nine of which it was raised, and in four of these it was more than 2·0 grammes *per centum*. In two cases the concentrations were at the upper limit of normal, 1·46 and 1·47 grammes *per centum*. Nine of the 11 patients also had a serum β globulin concentration above 0·8 gramme *per centum*. In only one of these 11 patients was the serum albumin level less than

3·0 grammes *per centum*. The serum total protein was elevated in 11 of the 35 cases in which it was determined, and depressed in one.

Continuing Hepatitis

Under the heading of continuing hepatitis we have placed 30 patients, in 28 of whom liver biopsy showed the presence of active

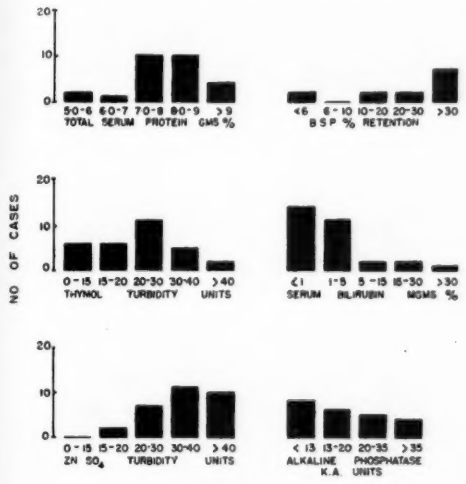
NO OF CASES
TOTAL SERUM PROTEIN GMS %
B S P % RETENTION
THYMOL TURBIDITY UNITS
SERUM BILIRUBIN MGMS %
ZN SO4 TURBIDITY UNITS
ALKALINE PHOSPHATASE K.A. UNITS

FIGURE II

The distribution of the results of six liver function tests in 30 cases of continuing hepatitis

hepatitis with early formation of fibrous tissue in and around the liver lobules, and two had undoubted clinical evidence of chronicity. All these patients had a definite history of acute hepatitis within the preceding three years.

The data from these patients are expressed graphically in Figure II.

The zinc sulphate turbidity test gave a positive result in every case, and in 21 the reading was greater than 30 units. In contrast, the thymol turbidity test produced negative results in six cases, and in six there was a small rise of up to 20 units. The bromsulphthalein retention was estimated in 13 cases; it was normal in two, more than 10% in 11, and more than 30% in seven. The serum alkaline phosphatase level was normal in eight of the 22 cases in which it was determined, in four it was above 35 King-Armstrong units, and in two of the latter there was evidence of cholangiolitic hepatitis. The serum bilirubin level was raised in just over half of these cases, and in three the level was more than 15 milligrammes per 100 millilitres. The serum γ globulin concentration was above 1·8 grammes *per centum* in all the nine cases in which it was determined; two patients had levels between two and three grammes *per centum*, and three had levels greater than four grammes *per centum*. The serum β globulin concentration was above 0·8 gramme *per centum* in five of the nine cases, and the serum albumin concentration was below three grammes *per centum* in seven of them. The serum total protein concentration was increased in 14 of 27 cases.

Five of these patients died, and in three of these the thymol turbidity was greater than 25 units and the zinc sulphate turbidity was greater than 35 units on their admission to hospital.

Cirrhosis

Thirty-four of the 41 patients with cirrhosis studied had liver biopsies and histological evidence of the disease (Figure III). The

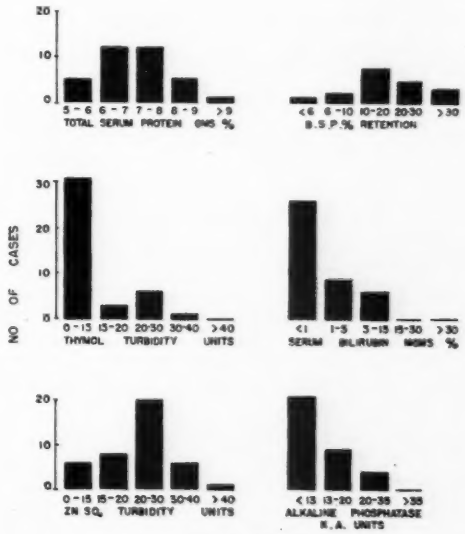

FIGURE III

The distribution of the results of six liver function tests in 41 cases of cirrhosis

remaining seven patients had gross evidence of cirrhosis, since four had ascites, and three had ascites, œsophageal varices and other less definitive signs such as "spider nævi" and "liver palms". Five of these latter seven patients had long alcoholic histories, three had previously been admitted to hospital for beriberi, and two had cardiac cirrhosis due to long-standing tricuspid incompetence.

The zinc sulphate turbidity test gave a positive result in 35 out of these 41 cases, but the thymol turbidity was increased in only 10. The bromsulphthalein retention was increased in 18 of the 19 cases in which it was estimated, and the majority of patients retained more than 10% of the dye. The serum alkaline phosphatase concentration was normal in two-thirds of the 34 estimations, and no patient had a level greater than 35 King-Armstrong units, as is found in obstructive jaundice. Jaundice was present in less than half the patients, and the serum bilirubin concentration was never over 12 milligrammes per 100 millilitres. The serum γ globulin concentration was estimated in six cases and was above two grammes *per centum* in all of them, and in two it was over three grammes *per centum*. The serum β globulin concentration was elevated above 0·8 gramme *per centum* and the serum albumin concentration was below 2·8 grammes *per centum* in all six cases. The serum total protein was determined in 35 cases and was normal in the majority.

Thirteen of these patients died, nine of them had jaundice on their first admission to hospital, 10 gave normal results to thymol turbidity tests, and five of the six who had their bromsulphthalein retention determined retained more than 20% of the dye.

Extrahepatic Biliary Obstruction

Of the 15 patients with extrahepatic biliary obstruction (Figure IV), three were not jaundiced at the time of the initial examination, but they had all had an episode of jaundice within the three weeks prior to their admission to hospital, due to common bile duct calculi in two cases and to a papilloma of the ampulla of Vater in one.

Three patients had obstruction for more than eight weeks, and each of them gave positive results to zinc sulphate and thymol turbidity tests. The remaining 12 patients gave negative results to both zinc sulphate and thymol turbidity tests. The bromsulphthalein retention of only two patients was estimated, both of them being jaundiced, and the figures of 55% and 70% were obtained. The serum alkaline phosphatase level was normal in two jaundiced patients, in seven it was raised, but less than 35 King-Armstrong units, and six had levels of over 35 King-Armstrong units. Of 12 jaundiced patients, six had serum bilirubin levels between 5·0 and 15·0 milligrammes per 100 millilitres, but none had bilirubin levels greater than 30 milligrammes per 100 millilitres. The serum γ globulin concentration of five of the 15 patients was determined. In two it was above 1·5 grammes *per centum* (1·9 and 1·62 grammes *per centum*), and in both the obstruction was of long standing and the zinc sulphate and thymol turbidities were increased. The remaining three patients had levels between 1·0 and 1·4 grammes *per centum*. The serum total protein level was determined in 12 cases and was normal in the majority.

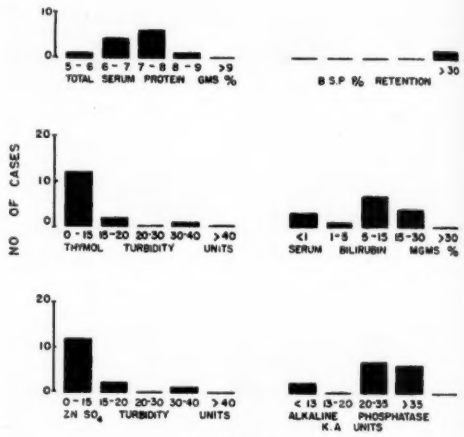

FIGURE IV

The distribution of the results of six liver function tests in 15 cases of extrahepatic biliary obstruction

Secondary Neoplasm of the Liver

There were 11 patients with secondary neoplasm of the liver, seven of whom had secondary carcinoma from breast, stomach or large bowel, two abdominal Hodgkin's disease, one a neuroblastoma of the liver and one had secondary melanomatous deposits. The zinc sulphate turbidity was raised in six of these cases, but in three of them it was below 20 units and in three between 20 and 30 units. The thymol turbidity was increased in four of the nine cases, but only just above the normal range. The serum alkaline phosphatase was greatly raised in nine of 11 cases, and in all but two of these the levels were greater than 35 King-Armstrong units. The bromsulphthalein retention was estimated in six cases; in all it was above 10%, and in three above 30%. The serum bilirubin level was raised in only one case, being 23 milligrammes per 100 millilitres.

Hæmochromatosis

Five patients with hæmochromatosis were studied, and the zinc sulphate turbidity was

TABLE II

Table Showing the Results of Liver Function Tests in Cases of the Miscellaneous Group

Disease	Number of Cases	Liver Function Tests[1]																					
		Zinc Sulphate Turbidity			Thymol Turbidity			Bromsulphthalein Retention			Serum Alkaline Phosphatase			Serum Bilirubin			Globulin			Serum Total Protein		
Fatty infiltration	3	+	+	−	+	−	−	+	+	−	−	−	+	+	−	−	+	+	0	+	−	−
Extrahepatic portal obstruction	3	−	−	−	−	−	−	+	+	0	+	−	0	−	−	−	0	0	0	−	−	0
Cincophen poisoning	2	+		−	−		−	0		0	0		−	−		+	+		0	+		+
Homologous serum hepatitis	2	+		+	+		+	0		0	+		+	+		+	0		0	+		−
Gaucher's disease	2	+		+	+		+	0		0	0		0	−		−	+		0	+		0
Multiple hydatid cysts	2	+		+	−		−	+		0	+		+	+		−	0		0	0		−
Porphyria	1	+			−			0			0			−			0			−		
Polyarteritis nodosa	1	+			−			0			−			−			0			+		
Myelomatosis	1	+			−			0			−			−			0			+		
Cholangitis	1	+			+			0			+			−			0			−		
Leuchæmia	1	+			−			+			0			+			0			−		
Follicular lymphoblastoma	1	−			−			−			0			−			+			+		
Myeloid metaplasia	1	+			+			+			−			−			0			−		
Exfoliative dermatitis	1	+			−			−			−			−			0			+		
Multiple abscesses	1	−			−			+			−			−			0			+		
Tuberculous hepatitis	1	−			−			0			+			+			−			+		
Total	24	24			24			11			17			24			6			21		
Number of positive findings	—	16			7			8			8			7			5			11		

[1] " + " indicates a positive or abnormal result, " − " indicates a negative or normal result, " 0 " indicates that the test was not performed.

increased in three cases, in two of which it was above 30 units. The thymol turbidity was increased to just above the upper limit of normal in one case. One patient was significantly jaundiced, and two had a small increase in the bromsulphthalein retention. One had a raised serum alkaline phosphatase level, and all had a total serum protein content between seven and eight grammes *per centum*.

Miscellaneous Group

The 24 cases in the " miscellaneous " group are shown in Table II, and in 21 of them liver biopsies were taken.

Fatty Infiltration of the Liver

All three patients with fatty infiltration of the liver had a long alcoholic history, and the only abnormal liver function findings were raised zinc sulphate turbidity and thymol turbidity in one case, and an increased bromsulphthalein retention in two cases.

Extrahepatic Portal Obstruction

There were three patients with extrahepatic portal obstruction. At operation all three were found to have portal hypertension with obstruction of the portal vein, and normal liver tissue was removed at biopsy. None of them had abnormalities of zinc sulphate turbidity, thymol turbidity, serum alkaline phosphatase or serum bilirubin concentration, but two had increased bromsulphthalein retention.

Patients Presenting with Jaundice

There were 87 patients who had clinical jaundice on their admission to hospital, the classification of whom into medical and surgical groups was assisted by liver function tests. Of the 15 patients with extrahepatic biliary obstruction, 12 gave low normal results to flocculation tests with normal serum protein patterns and elevated serum alkaline phosphatase levels. In three cases in which the obstruction was of long duration, the zinc sulphate turbidity and thymol turbidity were raised. On the other hand, all but two of 72 patients with a hepatocellular jaundice gave positive results to flocculation tests, and in two exceptions the ætiological agents were cincophen and miliary tuberculosis respectively.

INDIVIDUAL TESTS

A comparison of the frequency of positive results in the various tests is shown in Table III.

Zinc Sulphate Turbidity Test

The zinc sulphate turbidity was raised in 136 out of 170 cases of liver disease. It was positive in every case of continuing hepatitis, in all except one case of acute hepatitis (and

TABLE III

A Comparison of the Results of the Liver Function Tests in Various Liver Diseases

Disease	Number of Cases	Liver Function Tests													
		Zinc Sulphate Turbidity		Thymol Turbidity		Bromsulphthalein Retention		Serum Alkaline Phosphatase		Serum Bilirubin		γ Globulin		Serum Total Protein	
		Number of Tests Performed	Number of Positive Results	Number of Tests Performed	Number of Positive Results	Number of Tests Performed	Number of Positive Results	Number of Tests Performed	Number of Positive Results	Number of Tests Performed	Number of Positive Results	Number of Tests Performed	Number of Abnormal Results	Number of Tests Performed	Number of Abnormal Results
Acute hepatitis	44	44	43	44	38	3	1	41	26	44	35	11	9	35	12
Continuing hepatitis	30	30	30	30	24	13	11	23	17	30	16	9	9	27	16
Cirrhosis	41	41	35	41	10	19	18	35	14	41	15	6	6	35	11
Extrahepatic biliary obstruction	15	15	3	15	3	2	2	15	13	15	12	5	2	15	13
Secondary neoplasm	11	11	6	11	4	7	7	11	9	11	1	1	1	8	3
Hæmochromatosis	5	5	3	5	2	3	2	4	3	5	2	1	0	3	3
Miscellaneous	24	24	16	24	7	11	8	17	8	24	7	6	5	21	11
Total	170	170	136	170	88	58	49	146	90	170	88	39	32	138	59
Percentage of positive results	—	—	80[1]	—	52[1]	—	84	—	65	—	52	—	82	—	43

[1] The reliability of the zinc sulphate turbidity and thymol turbidity tests is understated. It actually is 85% and 57% respectively, as the turbidity is normally decreased in patients with extrahepatic biliary obstruction (*vide* text).

in this case the result was positive later), and in all but six of 41 cases of cirrhosis. One of these six patients had the bromsulphthalein retention estimated and it was normal, three had abdominal paracenteses prior to collection of the blood sample, one was in hepatic coma, and the disease was clinically and histologically inactive in the remaining two patients.

The zinc sulphate turbidity was raised in three cases in which extrahepatic biliary obstruction had been present for more than two months, and it increased on relief of the obstruction. It rose post-operatively to abnormal levels in two cases in which the pre-operative readings were in the low normal range. It was raised in six cases in which there were secondary neoplastic deposits in the liver but without any evidence of cirrhosis, but it was also raised in three of five cases in which hæmochromatosis was present, and all had histological evidence of cirrhosis. In the "miscellaneous" group the turbidity was increased in more than half, and this was the only positive finding in six cases.

The zinc sulphate turbidity (and the γ globulin estimation when this was made) was the only positive finding in 21 of 170 cases, and in 16 it was the only indication of liver disease other than the presence of jaundice.

Nine patients, comprising four with secondary neoplasm of the liver, two with extrahepatic portal obstruction and one each with hæmochromatosis, fatty infiltration of the liver and multiple liver abscesses, showed a normal response to the zinc sulphate turbidity test but an increased bromsulphthalein retention. Twenty-five patients gave negative results to the zinc sulphate turbidity test, and 12 patients gave normal results to all the tests.

Thymol Turbidity Test

The thymol turbidity was increased in 97 out of 170 cases of liver disease. In acute hepatitis its result was normal in six of 44 patients, in one of which it later became positive, in continuing hepatitis the result remained constantly negative in six of 30 cases, and of 41 cases of cirrhosis it was positive in 10, and six of these ended fatally. In the miscellaneous group, in seven cases the result was positive, and in each of these the zinc sulphate turbidity test also produced a positive result.

Bromsulphthalein Retention

The bromsulphthalein retention was increased in the majority of cases of continuing hepatitis and cirrhosis. In one case of inactive cirrhosis, portal hypertension and œsophageal varices its result was negative, as were the results of both flocculation tests. The result was positive in every case of secondary neoplasm of the liver, and all these patients retained more than 10% of the dye. The result was negative in one case of hæmochromatosis and

gross hepatic fibrosis. In the miscellaneous group of liver diseases the result was positive in involvement of the liver with fat, myeloid and leukæmic deposits, hydatid cysts and multiple abscesses, and in two cases of extrahepatic portal obstruction with normal histological findings in the liver.

Nine of 58 patients whose bromsulphthalein retention was estimated gave normal results; one of these nine had no other positive test findings, but seven gave a positive result to the zinc sulphate turbidity test, and two gave positive results to the thymol turbidity test as well. Of these seven patients, two had acute hepatitis, two had continuing hepatitis, and the remaining three had cardiac cirrhosis, exfoliative dermatitis and fatty infiltration respectively. In four of the 49 cases in which the bromsulphthalein retention was abnormal, it was the only positive finding (secondary neoplasm of the liver, hæmochromatosis, multiple liver abscesses and extrahepatic portal obstruction).

Serum Alkaline Phosphatase Concentration

The serum alkaline phosphatase concentration was increased in two-thirds of the patients who had acute hepatitis and continuing hepatitis, and usually afforded evidence of the degree of intrahepatic biliary obstruction. However, in several cases it remained at very high levels without apparent significance. It was elevated in one-third of the cases of cirrhosis, but this elevation was often transient. In extrahepatic biliary obstruction it was normal in two cases in which the obstruction had been present for less than three days. It was normal in two patients of secondary neoplasm in which the bromsulphthalein retention was increased. In six of the remaining nine cases it was above 35 King-Armstrong units, but only one of these patients was jaundiced. In the miscellaneous group it was increased in one-half of the cases in which it was determined.

Serum Bilirubin Level

The serum bilirubin level was determined in all cases, and was raised in approximately one-half. In acute hepatitis the majority of patients had levels between 5·0 and 15 milligrammes per 100 millilitres, while in continuing hepatitis slightly less than half the patients had normal levels. Cirrhotic patients generally were not jaundiced on their admission to hospital.

While no patient with extrahepatic biliary obstruction had a serum bilirubin level above 30 milligrammes per 100 millilitres, three patients with hepatitis had levels above 40 milligrammes per 100 millilitres. This tends to confirm the statement of Ducci (1951) that a serum bilirubin level of more than 30 milligrammes per 100 millilitres indicates that "we are almost surely dealing with a hepatic kind of jaundice and not an obstructive one".

Serum γ Globulin Concentration

The serum γ globulin concentration was determined in 39 cases, and correlated reasonably well with the zinc sulphate turbidity. The results of 85 determinations in 39 cases are shown graphically in Figure V. The upper limit of the

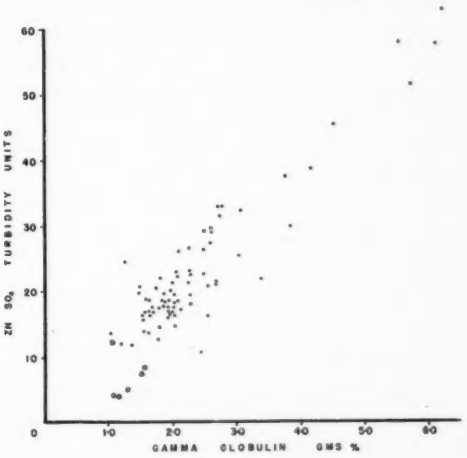

FIGURE V

Graph showing the correlation between the zinc sulphate turbidity and the concentration of the serum γ globulin in grammes *per centum*, representing 85 determinations on 40 patients with liver disease. The open circles represent the results of six determinations on patients with obstructive jaundice

normal value of zinc sulphate turbidity was, by chance, ten times the upper limit of the normal value for γ globulin in grammes *per centum* and in most cases the γ globulin was reasonably accurately determined by estimating the zinc sulphate turbidity. It is apparent that four or five patients with obstructive jaundice have zinc sulphate turbidities which are disproportionately low for the corresponding value of the γ globulin.

DISCUSSION

Liver function tests must always be correlated with the clinical assessment of the patient in the differential diagnosis of liver disease, and liver biopsy may be necessary before a final diagnosis is reached.

The tests will give almost 100% accuracy in cases of extrahepatic biliary obstruction if they are carried out early in the course of the disease, but the accuracy is decreased the longer obstruction has been present.

In 80% of our cases of extrahepatic biliary obstruction the zinc sulphate and thymol turbidity tests gave negative results, the serum alkaline phosphatase levels were greater than 30 King-Armstrong units, and there was minimal disturbance of total serum proteins. The serum bilirubin level was elevated, but was invariably less than 30 milligrammes per 100 millilitres.

It has been pointed out by Mawson (1948) and by Ducci (1951) that the mean value for the flocculation tests is lower in patients with obstructive jaundice than in normal patients, and it appears (Maclagan and Bunn, 1947; Popper *et alii*, 1950), that albumin may be a stabilizing factor tending to prevent flocculation. Ducci (1950), on the other hand, has shown that there is some other factor in the serum of patients with obstructive jaundice which prevents flocculation, even in the presence of abnormal protein fractions which produce flocculation in hepatocellular disease. Thus, absence of flocculation does not indicate the absence of hepatocellular damage; indeed, the results of these tests may become positive on relief of the obstruction.

In view of these facts, the presence of positive results to flocculation tests in extrahepatic biliary obstruction has an especial significance. It seems probable that such patients would be more likely to develop hepatic failure as a result of operative interference.

In hepatocellular disease the zinc sulphate and thymol turbidities are usually increased; the serum alkaline phosphatase level varies considerably, but may be in the " obstructive " range of 35 King-Armstrong units or more. The serum bilirubin content varies within a wide range and sometimes exceeds 40 milligrammes per 100 millilitres.

In acute infective hepatitis both the zinc sulphate and thymol turbidities are increased, but the former test is more reliable. The total serum proteins are often in the high normal range, not uncommonly over eight grammes *per centum*, and the greater part of this increase is due to γ globulins and the lesser to β globulin. There is often a decreased serum albumin concentration, and Maclagan and Bunn (1947) suggest that there is a qualitative alteration in the serum albumin in acute infective hepatitis which lowers its stabilizing effect on the flocculation tests. This provides one explanation for the common finding that the zinc sulphate turbidity is considerably increased though the serum albumin content is normal and the γ globulin only slightly elevated.

Liver function tests are most useful in assessing the progress of acute infective hepatitis, as the diagnosis is usually made on clinical grounds without much difficulty. It is our practice to carry out serial determinations in these cases to determine the amount of activity that may be permitted.

In our experience the zinc sulphate turbidity remains increased in the majority of cases long after all other test results have returned to normal, though it must be stressed that we have not carried out many bromsulphthalein estimations in these cases. However, we have seen a number of patients who had clear-cut histories of acute hepatitis, who presented with vague symptoms or simply for assessment, and whose evidence of hepatic disease was limited to a positive response to the zinc sulphate turbidity test and some hepatic enlargement or tenderness. Some of these patients have had bromsulphthalein retention estimated and found to be normal. Whilst it is impossible to be dogmatic as to the significance of an increased zinc sulphate turbidity in these cases, we do consider it to be an indication to retain restrictions.

Biochemical tests of hepatic function show obvious changes in the patient who fails to recover from the acute stage and shows evidence of continuing hepatitis. The zinc sulphate turbidity remains increased, the thymol turbidity and the serum alkaline phosphatase usually fall to near normal levels as the activity of the disease decreases, and the serum total protein level usually rises. There is a further rise in γ globulin concentration, and the serum albumin content falls. Bromsulphthalein retention is an accurate indication of the degree of liver damage.

In cases of continuing hepatitis serial tests are of great value. We have found the data from these tests to be of great assistance in determining prognosis, and particularly the optimal time at which a venous shunt operation may be performed. We defer operation until the results of flocculation tests suggest that the disease is inactive. The most significant change indicating cessation of the active process is the decrease in thymol turbidity with little change in zinc sulphate turbidity or bromsulphthalein retention. When the disease ultimately subsides, the final picture becomes biochemically and pathologically indistinguishable from that of cirrhosis.

Figure VI shows such changes in a woman, aged thirty-four years, with continuing hepatitis, who developed recurrent hæmatemeses as her hepatitis subsided, and ultimately had a splenorenal anastomosis carried out with complete relief of her bleeding.

In cases of cirrhosis the zinc sulphate turbidity and the bromsulphthalein retention are the most helpful tests, although we did not reproduce Kunkel's (1947) 100% accuracy with the former. We agree with Maclagan (1951) that some patients with cirrhosis, histologically shown to be inactive, do not give positive results to the zinc sulphate turbidity

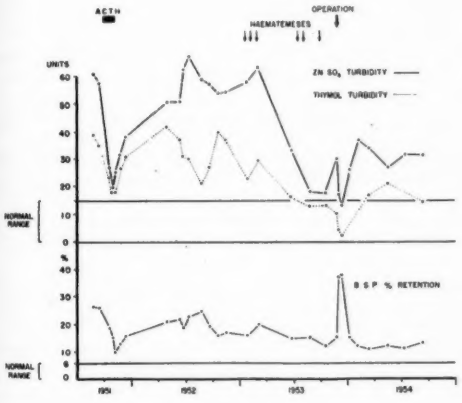

FIGURE VI

Graph illustrating the use of serial tests in timing the surgical treatment of portal hypertension in a patient with continuing hepatitis. The graph also shows the effect on the tests of a short course of ACTH

test, even though electrophoretic separation shows a lowered serum albumin content and a raised serum γ globulin concentration. The thymol turbidity is not often increased, in contrast to acute infective hepatitis and continuing hepatitis.

Increased zinc sulphate turbidity and bromsulphthalein retention are compatible with inactive cirrhosis. We regard the changes in serum total protein, thymol turbidity and serum bilirubin level as indicating active disease, and as indications for bed rest.

It has been suggested by Magath (1951) that patients with secondary carcinoma of the liver frequently have increased bromsulphthalein retention, and this has been our experience, since it was found each time it was estimated. The serum alkaline phosphatase test gave a positive result in nine out of 11 cases, but the results of other tests were usually negative, so that a combination of increased bromsulphthalein retention and a raised serum alkaline phosphatase content without other positive findings was suggestive of secondary neoplasm of the liver. Such findings would suggest a search for metastases in the liver by needle biopsy or exploratory laparotomy before surgical treatment of the primary growth is carried out.

Our results in cases of hæmochromatosis confirm Althausen's (1951) view that the liver function tests are disappointing, for they all produce negative results in about half the cases even in the presence of gross liver disease.

In the miscellaneous group of disorders the results of the zinc sulphate turbidity and bromsulphthalein retention tests were the most reliable indicators of liver disease. They were of most value in indicating hepatic involvement in generalized diseases such as leuchæmia, acute bacterial endocarditis and exfoliative dermatitis (Bauer, 1953).

The zinc sulphate turbidity and bromsulphthalein retention were found to be significantly abnormal more frequently than any other test of liver function in this series of cases, and they are considered to be the best screening tests for liver disease. Though there are more false positive results with the zinc sulphate turbidity test, it is the preferred all-purpose test on the grounds of its ease of performance and its cheapness. It cannot be substituted for the estimation of bromsulphthalein retention, or for any other test, except for the electrophoretic estimation of serum γ globulin.

The good correlation between the results of the zinc sulphate turbidity test and the electrophoretic estimation of serum γ globulin concentration (Discombe *et alii*, 1954) clearly renders the latter laborious determination unnecessary, and we no longer use it for patients with liver disease except for special purposes. In an occasional case it may be necessary if the result of the zinc sulphate turbidity test is normal in spite of clinical evidence of cirrhosis. It is seldom that electrophoresis is needed for the separation of patients with myelomatosis or nephrosis from those with liver disease.

The thymol turbidity test has its chief value in confirming the presence of active hepatocellular disease (Popper *et alii*, 1949), and therein differs from the zinc sulphate turbidity test in many instances. Its main use is in the diagnosis and follow-up investigation of patients with hepatitis and cirrhosis.

The total serum protein concentration should always be estimated, as it yields useful information, and if the falling-drop method is used it is easy, rapid and requires little serum.

These three tests—the zinc sulphate turbidity test, the thymol turbidity test and the serum total protein concentration test—will usually permit the separation of patients with acute infective hepatitis, continuing hepatitis and cirrhosis, and will be adequate for most purposes in their follow-up.

The estimation of bromsulphthalein retention is perhaps the most reliable test of hepatic function. It is of special value in the diagnosis and follow-up investigation of patients with relatively inactive liver disease in whom slow changes may be taking place. Its importance in secondary neoplasm has been mentioned.

It is suggested that the routine tests for patients in whom liver disease is present or suspected, should be the zinc sulphate turbidity test, the thymol turbidity test and estimation of the total serum protein concentration, while estimations of bromsulphthalein retention, serum alkaline phosphatase and bilirubin level are of great value in selected cases. Electrophoretic separation of serum proteins, except in a few cases, is not considered to yield information unobtainable by the determination of the zinc sulphate turbidity and the serum total proteins.

SUMMARY

Liver function tests, including serum protein electrophoresis, carried out in 170 cases of proved liver disease have been analysed.

Both the zinc sulphate turbidity test and the estimation of bromsulphthalein retention gave positive results in approximately 85% of cases, and were the most accurate indicators of the presence of liver disease.

Of 87 cases in which jaundice was a diagnostic problem, the zinc sulphate turbidity and thymol turbidity tests together were decisive in differentiating between hepatocellular and obstructive causes in 82. They were also of value in indicating the activity of the disease and the optimal time for surgical measures.

Of 11 cases of secondary neoplasm of the liver, the serum alkaline phosphatase content was increased in nine. Bromsulphthalein retention was measured in seven, and in all it was increased.

The value of the zinc sulphate turbidity test has been emphasized, and it is considered the most generally useful single liver function test.

Electrophoretic separation of serum proteins, except in a few cases, gives no more information than the determination of the zinc sulphate turbidity and the total serum proteins.

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THE VECTORELECTROCARDIOGRAM IN BUNDLE BRANCH BLOCK[1]

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SOMETIMES, particularly in the presence of myocardial disease, the electrocardiogram shows *QRS* complexes which are of increased duration and bizarre shape. Because the increased duration suggests an abnormal route of spread of the electrical activity over the heart, and because there is a marked resemblance between many of these complexes and those seen in animal experiments in which the branch-conducting bundles were divided, these complexes are said to arise from blocking of the branch bundles or their arborizations.

These abnormal complexes may be divided into two groups: first, complete bundle branch block; secondly, incomplete or arborization block. Right and left bundle branch block resemble the corresponding animal tracings, and show respectively delay of excitation over the right or left ventricle in the precordial leads. Incomplete and arborization blocks do not show these complete pictures.

Correlation between the electrocardiographic pattern and histological autopsy evidence of actual bundle damage has not been frequently obtained in man; but Lenègre (1952) claims to have demonstrated this correlation in a series of 46 cases.

The pattern of bundle branch block is therefore an electrocardiographic pattern. It is seen usually in damaged hearts, but has as yet no clearly defined anatomical or physiological basis. This pattern contains disturbances of time relationship and contour of the various parts of the ventricular electrical complex.

In view of the greater ease of analysis of the *QRS-T* complex by a high quality spatial vectorelectrocardiogram, this method has been used to study a series of patients whose electrocardiograms have shown right or left bundle branch block patterns.

CLINICAL MATERIAL AND METHODS

Patients were selected for this study solely on the basis that electrocardiograms taken in routine studies showed the accepted pattern of right or left bundle branch block. These criteria (Myers, 1950) may be summarized as follows:

Right bundle branch block:

1. Duration of *QRS* complex greater than 0·11 second.
2. Initial upstroke in all leads facing epicardial surface of right ventricle.
3. Late secondary upstroke in all leads facing epicardial surface of right ventricle.

Left bundle branch block:

1. Duration of *QRS* complex greater than 0·11 second.
2. Initial upstroke in all leads facing epicardial surface of left ventricle.
3. Broad, slurred or notched *R* wave with abnormally delayed intrinsicoid deflection in those leads facing left ventricle.

TABLE I

Conditions for which Patients were being Treated

Conditions	Right Bundle Branch Block: Number of Patients	Left Bundle Branch Block: Number of Patients
Congenital heart disease:		
Atrial septal defect 4		
Ebstein's anomaly 2	6	—
Hypertension	4	5
Angina pectoris	1	2
Hypertension and *angina pectoris*	4	4
Myocardial infarction	1	3
Congestive cardiac failure	—	1
Emphysema	1	—
Depressed sternum	1	—
Carcinoma of the œsophagus	1	—
Left mammary pain	1	—
Acute nephritis	—	1
Osteoarthritis	—	1
Cardiac hypertrophy	—	2
Peripheral arterial disease	—	1

There were 20 patients in each series, and Table I shows the clinical conditions for which they were being treated.

The ages of the patients with right bundle branch block were between sixteen and seventy-nine years, with an average of 48·5 years, and of those with left bundle branch block between forty-six and eighty-one years, with an average of 62·5 years.

Spatial vectorelectrocardiograms were made with the apparatus previously described (Lowe

[1] Received on June 21, 1954.

[2] Part of the expenses of this investigation have been defrayed by a grant from the National Health and Medical Research Council.

and Goble, 1952). Frontal, sagittal and horizontal projections of the spatial loop were photographed in pairs simultaneously. The orientation of the projection is shown in Figure I. High amplification tracings were used to clarify events about the origin.

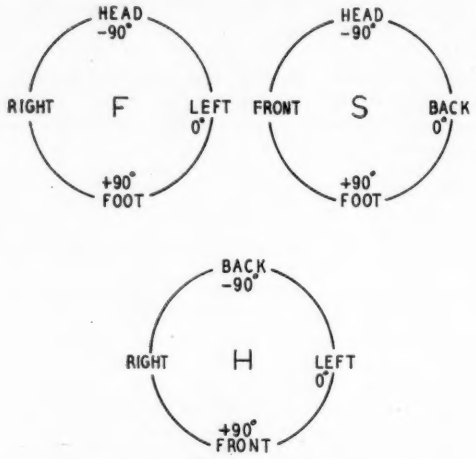

FIGURE I

Diagram to show the orientation of the vectorelectrocardiogram loops in all illustrations

RIGHT BUNDLE BRANCH BLOCK

Duration of the QRS Loop

In this series the duration of the *QRS* loop ranged from 0·115 to 0·17 second. The increased time of inscription was due in all cases to a slowing in the terminal third of the *QRS* loop. In all these cases the duration of the *QRS* complex in the scalar electrocardiogram was 0·12 second or greater.

TABLE II

Direction of Inscription of QRS Loop

Direction of Inscription	Right Bundle Branch Block	Left Bundle Branch Block
Sagittal:		
Normal	8	18
Abnormal	12	2
Horizontal:		
Normal	7	1
Abnormal ..	13	19

Direction of Inscription of the QRS Loop

In the normal vectorelectrocardiogram the sagittal and horizontal loops are written anticlockwise. In the right bundle branch block series (see Table II) the sagittal loop was written clockwise in 12 cases, and the horizontal loop was written clockwise in 13. In the remainder the direction of inscription was normal.

Direction of the Long Axis of the QRS Loop

The histogram (right bundle branch block) in Figure II shows the direction of the long axis of the *QRS* loop as recorded in each of the three projections, and beneath it are given the limits and the median of the directions found in the normal series previously reported (Lowe and Goble, 1952).

It will be seen that in all projections most of the patients had loops with axes in the normal direction, but that in the frontal projection 8/20 of the axes were shifted to the left, in the sagittal projection 9/20 of the axes were shifted up, and in the horizontal projection 1/20 of the axes were shifted back and 2/20 forwards.

Abnormality of direction of the long axis of the *QRS* loop is apparently not an essential feature of the pattern of right bundle branch block, but is sometimes seen.

Contour of the QRS Loop

In all cases of the series the contour of the *QRS* loop was abnormal (see Table III), and it is possible to divide the abnormalities into two groups. First, there are the abnormalities which correspond to recognizable conditions other than right bundle branch block. Thus

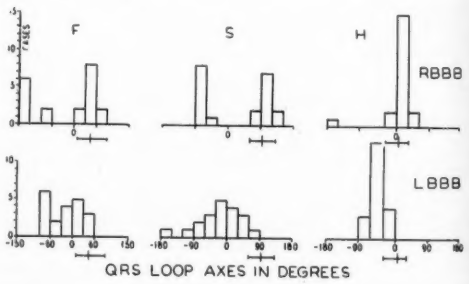

FIGURE II

Histograms showing the frequency distribution of the *QRS* axis angles in frontal (F), sagittal (S) and horizontal (H) planes. Beneath each histogram are given the range and median of the axis angle in a series of normal subjects; R.B.B.B., right bundle branch block; L.B.B.B., left bundle branch block.

there were six cases in which the loop resembled the "fling up" type of left ventricular hypertrophy (Gardiner and Lowe, 1953) (Figure III) and three which resembled that of right ventricular hypertrophy (Gardiner and Lowe, 1954) (Figure IV), five were bizarre loops which have been previously commented upon (Gardiner and Lowe, 1953), but of which the pathogenesis is uncertain, and one showed the pattern of myocardial infarction (Figure V). In the remaining cases the loops showed only the

abnormality of right bundle branch block, being otherwise normal in contour (Figure VI).

Secondly, in all cases, the contour of the centripetal limb in its later parts was abnormal.

TABLE III

Analysis of Right Bundle Branch Block Loop Types and the Direction of Displacement of Centripetal Limb Relative to Origin

Displacement Relative to Origin	Type of Loop				Total
	Fling Up (6)	Right Ventricular Hypertrophy (3)	Bizarre (5)	Simple Right Bundle Branch Block (6)	
Right	6	3	5	6	20
Central	—	—	—	—	—
Left ..	—	—	—	—	—
Up ..	2	1	4	3	10
Central	1	—	—	—	1
Down ..	3	2	1	3	9
Forward	3	2	3	3	11
Central	3	—	—	1	4
Back ..	—	1	2	2	5

It ended to the right of the origin (*O*) and was followed by a slow run into *O* or *J*. This change seems to be the characteristic feature of the vectorelectrocardiogram in right bundle branch

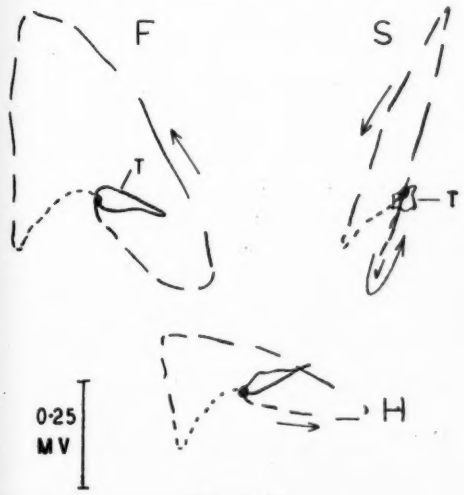

FIGURE III

Traced drawings of vectorelectrocardiogram in right bundle branch block with left ventricular hypertrophy. Time markers in all vectorelectrocardiograms are 0·006 second apart.

block. In one (Figure VII) of the six cases in which only this abnormality was present, autopsy showed no evidence of ventricular hypertrophy or coronary artery disease.

Displacement of the QRS-T Junction (J)

In 11 of the 20 cases the junction of the *QRS* and *T* loops did not coincide with *O*, and the direction of this *J* displacement is recorded in Table IV.

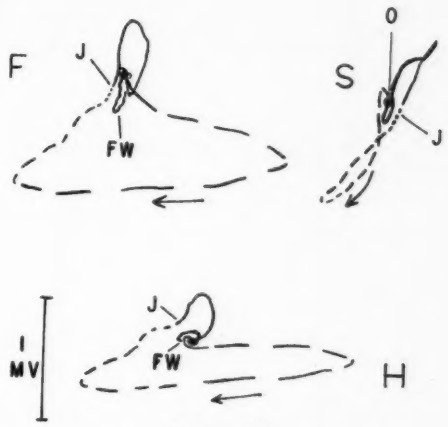

FIGURE IV

Traced drawings of vectorelectrocardiogram in right bundle branch block with right ventricular hypertrophy; Fw, flutter waves.

It will be noted that there is no uniformity in the direction of this displacement. Of these 11 patients, two were considered to have a vectorelectrocardiographic pattern of left ventricular hypertrophy, three of right ventricular hypertrophy and one of myocardial infarction. All these conditions are frequently associated with *J* displacements. Four of the remaining five patients with *J* displacement were suffering from *angina pectoris*, and one had congenital heart disease, conditions which also are frequently associated with *J* displacement.

FIGURE V

Traced drawings of vectorelectrocardiogram in right bundle branch block with myocardial infarction.

It appears therefore that displacement of *J* is not a characteristic feature of the simple right bundle branch block pattern.

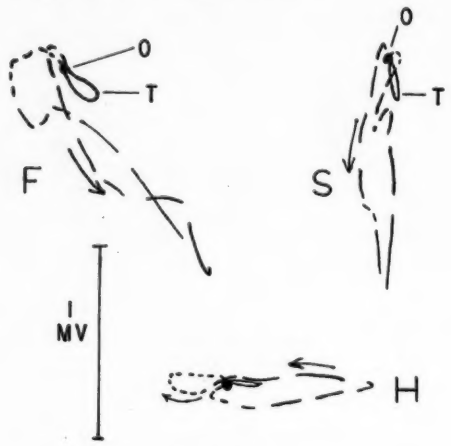

FIGURE VI
Traced drawings of vectorelectrocardiogram with uncomplicated right bundle branch block.

QRS-T Spatial Angle

The spatial angle between the long axis of the *QRS* and *T* or *J-T* segments was increased above the normal upper limit of 40° in 14 of the 20 cases. However, in eight of these 14 patients, as was mentioned above, a *J* shift

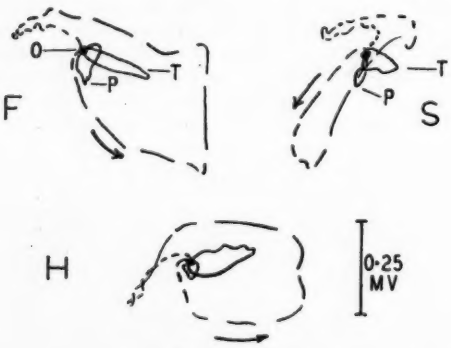

FIGURE VII
Traced drawings of vectorelectrocardiogram with uncomplicated right bundle branch block.

was present, and the increased angle might therefore have been due to conditions other than right bundle branch block. Of the remaining six patients, four were suffering from hypertension and two from congenital heart disease.

It would appear that increase in the *QRS-T* spatial angle is not necessarily a feature of right bundle branch block patterns.

LEFT BUNDLE BRANCH BLOCK

Duration of the QRS Loop

In the left bundle branch block series the duration of the *QRS* loop ranged from 0·105 to 0·185 second. In contrast to right bundle branch block, this increased duration was most frequently due to a general slowing of inscription throughout the whole cycle. The rate of inscription was often irregular. General slowing was seen in 14 of the 20 cases, but in a few of these the slowing was more pronounced in the second half of the loop. In three cases the slowing was entirely confined to the second half of the loop, and in another three it was seen only in the middle third of the loop.

TABLE IV
Direction of Displacement of J Relative to O

Direction	Number of Cases	
	Right Bundle Branch Block (11 out of 20)	Left Bundle Branch Block (20 out of 20)
Right	4	19
Central	4	1
Left	3	0
Front	0	20
Central	6	0
Back	5	0
Up	5	2
Central	4	9
Down	2	9

Direction of Inscription of the QRS Loop

In the sagittal projection, in contrast to the right bundle branch block cases, the direction of inscription was normal (anti-clockwise) in 18 of the 20 cases. However, in the horizontal projection the loop was abnormally inscribed (figure-of-eight) in 19 of the 20 (see Table II).

Direction of the Long Axis of the QRS Loop

The histogram (left bundle branch block) in Figure II shows the direction of the long axis of the *QRS* loop as recorded in each projection.

It will be noted that, in all projections, this axis is in the majority of cases abnormally directed, being displaced to the left in the frontal projection, upwards and backwards in the sagittal, and backwards in the horizontal. These displacements are in the same direction as those seen in left ventricular hypertrophy, although a greater proportion of the axes are

displaced than was the case in our series of patients with that condition. The concentration of values in the range −30° to −60° in the horizontal projection seems characteristic of left bundle branch block.

Contour of the QRS Loop

The contour of the *QRS* loop was abnormal in all cases, and all portions of the loop were affected. The initial crochet was absent in 15 cases (Figure VIII). In a further four it was abnormally placed, passing downwards, forwards and to the left. In one case it was normal. The centrifugal limb passed to the left and backwards, sometimes very much so.

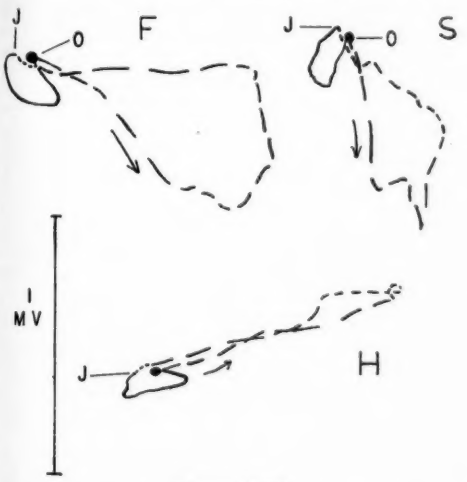

FIGURE VIII

Traced drawings of vectorelectrocardiogram with left bundle branch block. Note absence of initial crochet and narrow figure-of-eight horizontal loop.

From here the centripetal limb passed generally upwards and forwards before returning towards the origin, but passed to the right and usually above and in front of *O* to reach *J*.

A characteristic feature of the loops in this series is the narrow figure-of-eight loop (Figure VIII) in the horizontal projection directed to the region of −45°, and an openness of the frontal and sagittal loops which resemble each other in a general way (Figure IX).

It has not been possible in this series to identify clearly loop types characteristic of other associated changes, although six of the patients had hypertension, five *angina pectoris* and five myocardial infarction.

Displacement of the QRS-T Junction (J)

The *QRS-T* junction (*J*) was displaced from *O* in all cases, and Table IV records the direction of this displacement. It is, in most cases, to the right and forwards of *O* (Figure X), and either level with or below the origin.

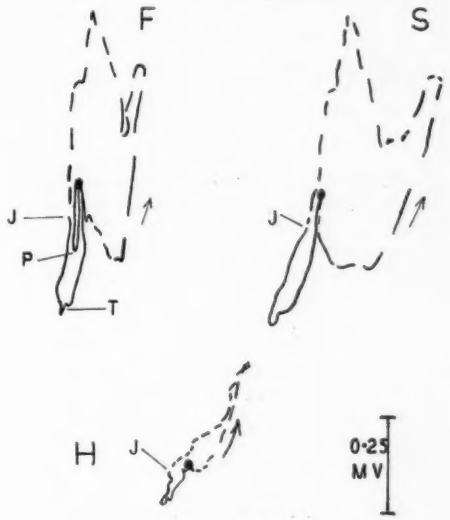

FIGURE IX

Traced drawings of vectorelectrocardiogram with left bundle branch block. Note resemblance of frontal and sagittal loops.

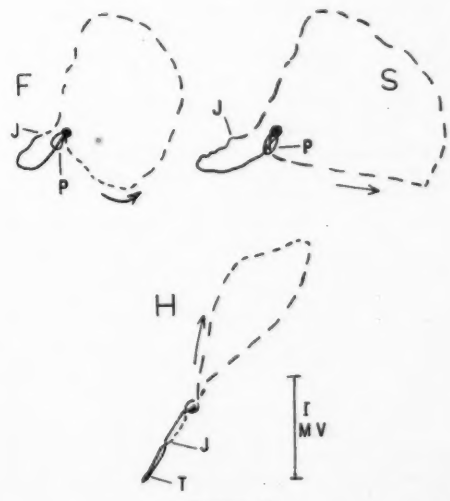

FIGURE X

Traced drawings of vectorelectrocardiogram with left bundle branch block. Note *J* displacement.

In contrast to right bundle branch block, displacement of *J* appears to be a regular feature of the pattern, and differs from that of left ventricular hypertrophy, in which the

displacement is to the right, forwards and upwards of *O*, and from that of right ventricular hypertrophy, which is usually to the right, backwards and upwards.

QRS-T Spatial Angle

The spatial angle between the long axes of the *QRS-T* loop was increased above normal in 19 of the 20 cases.

DISCUSSION

From this description of the vectorelectrocardiogram loops in bundle branch block a clear picture emerges. The loop characteristics are such that general inspection indicates whether the disturbance is that of a right or left bundle branch block.

In right bundle branch block the *QRS* loop is of increased duration owing to a slowing of inscription of the terminal part of the loop which runs into *O* or *J* from a point to the right of *O*. In left bundle branch block the *QRS* loop is also of increased duration, but is usually abnormal in all portions. The long axis of the loop is displaced to the left, upwards and backwards from its normal position. The horizontal loop axis is frequently directed backwards at an angle of $-45°$, and the loop is a thin figure-of-eight. The frontal and sagittal loops are open and resemble each other. *J* is usually displaced to the right and forwards.

The right bundle branch block deformity of the *QRS-T* loop occurs superimposed on other types of abnormality which may affect the earlier parts of the loop, and these other abnormalities can usually be identified. However, in left bundle branch block the whole loop is abnormal, and it is doubtful whether any other coexistent lesions can be identified.

The terminal slowing and the run-in to *O* or *J* from the right seen in right bundle branch block occurs sometimes in patients with right ventricular hypertrophy, but is then unaccompanied by an increased duration of the complex. We have not as yet any observations which enable us to state whether this change is part of that due to the right ventricular hypertrophy, or whether it is in fact due to a conduction defect.

The position in space of the loops seen in left bundle branch block and left ventricular hypertrophy is somewhat similar, but in the horizontal projection the loop of left bundle branch block is narrow and usually a figure-of-eight with the distal loop inscribed clockwise, whereas that of left ventricular hypertrophy is open and inscribed anti-clockwise.

The absent or abnormal initial crochet in the loop of left bundle branch block corresponds with the absence of a *Q* wave in scalar electrocardiogram tracings from leads over the left ventricle.

When the vector loops are unusually placed in space, the localized slowing of inscription in right bundle branch block and the general change in left bundle branch block may allow of a decision as to the type in a doubtful case, for they are not dependent on the position of the loops. This point is of some importance in the diagnosis of some cardiac congenital anomalies, and is more easily seen in a vectorelectrocardiogram than in a standard electrocardiogram.

SUMMARY

Twenty cases of right and 20 of left bundle branch block were selected from routine scalar electrocardiograms by standard criteria. Vectorelectrocardiograms were obtained from these patients by electronic synthesis.

In general the characteristic features of the vectorelectrocardiograms are as follows: (*a*) in right bundle branch block, an increased duration of the *QRS* complex with the increase due to a terminal slowing in inscription of the loop and a "run-in" to the origin from the right; (*b*) in left bundle branch block, an increased duration of the *QRS* complex due to general, often irregular, slowing in inscription of the loop. The horizontal projection of the *QRS* loop is figure-of-eight with the distal loop inscribed clockwise. The frontal and sagittal projections are open and have a general resemblance. The *QRS-T* junction is displaced to the right and forwards of *O*.

In right bundle branch block coexistent lesions can be identified in the patterns, but this cannot be done in left bundle branch block.

ACKNOWLEDGEMENTS

We wish to express our appreciation and thanks to the many colleagues and assistants who have made this investigation possible.

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LUMBAR SYMPATHECTOMY FOR OCCLUSIVE ARTERIAL DISEASE[1]

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LUMBAR SYMPATHECTOMY has long been used as treatment for peripheral arterial disease of the legs, and there are highly favourable reports of its value both in ischæmia of the skin and in intermittent claudication (Coller *et alii*, 1949; Edwards and Crane, 1951; Pearl and Rosenman, 1951). However, some workers have found it of no benefit to patients whose main symptom is claudication (Shepherd, 1950*a*; Hamilton and Wilson, 1952).

The main therapeutic effect of sympathectomy is reduction of arteriolar tone distal to an arterial block. This tone persists to some degree in all but the most severe forms of arterial occlusion. Whether benefit will follow the operation will depend on the amount of tone still present and the proportion it contributes to the regional peripheral resistance. Recent work indicates also that sympathectomy causes dilatation of collateral vessels (Shepherd, 1950*b*; Dornhorst and Sharpey-Schafer, 1951; Longland, 1953) and accelerates their growth, although it does not increase their ultimate extent.

Since the sympathetic transmitter, noradrenaline, causes constriction of both skin and muscle vessels (Barnett *et alii*, 1950), sympathectomy would be expected to improve the blood flow in both these tissues. However, although sympathetic nervous interruption increases the resting muscle blood flow, it does not increase the augmented blood flow from muscular exercise (Barcroft and Swan, 1953). Thus sympathectomy would be expected to relieve symptoms of skin ischæmia, but not necessarily intermittent claudication from muscle ischæmia.

The hyperæmia immediately after sympathectomy diminishes, at first rapidly and then more slowly; one to three months after operation the foot blood flow is still about double its pre-operative level (Lynn and Barcroft, 1950). The return of tone in sympathectomized vessels provides an argument against the operation being performed at an early stage in the disease. However, intrinsic tone in leg vessels develops relatively slowly, and in a subject studied by Lynn and Barcroft (1950) the resting blood flow was more than double the pre-operative value after nine months. Sympathectomy is indicated when the symptoms are severe enough to require relief.

CLINICAL DATA

MATERIAL

In 23 of the 30 cases forming the basis of this study, chronic ischæmia was due to *atherosclerosis obliterans*. The diagnoses in the other seven cases were *thromboangiitis obliterans* (two), acute arterial thrombosis (three), and thrombotic occlusion of the lower end of the aorta (two). The ages ranged from thirty-two to eighty-five years; 18 of the patients were in the sixth and seventh decades. There were 27 men and three women. Twenty-two of the patients had intermittent claudication and 28 "distal" symptoms (rest pain, paræsthesia, ulceration of the foot).

TABLE I

Follow-up Status[1]

Status	Number of Patients
Known dead	4
Interviewed	20 } 23
Replied to letter only	3 }
Others	3

[1] The period has varied from a few weeks to four years.

Letters were sent to the patients believed to be surviving, asking for details of their assessment of the effect of the operation on their walking distance and on other symptoms, and asking the patient to come for interview and examination. The status of the patients in respect to this follow-up (in April, 1954) is shown in Table I.

The study has been concerned with patients rather than with limbs. In those treated by bilateral synpathectomy, the results have in general been considered in terms of the worse limb.

[1] Read at a meeting of The Royal Australasian College of Physicians, May 27, 1954, at Melbourne.

RESULTS

Immediate Results

One patient died from enterocolitis with circulatory collapse two days after operation. As patients are unable to walk at the normal pace soon after operation, assessment of early symptomatic change has been limited to "distal" symptoms. The effect of operation on the 28 patients with these symptoms is summarized in Table II.

TABLE II

Early Effect of Lumbar Sympathectomy on "Distal" Symptoms	Number of Cases
Failure to halt progress of gangrene; amputation	3
Definite improvement	21
No significant early change	1
Not adequately assessed	3[1]
Total	28

[1] The patient who died soon after the operation with circulatory collapse is included here. The other two patients had only mild "distal" symptoms.

In the patients recorded as "improved" there were one or more of the following features: increased warmth of foot, improved colour, loss or diminution of rest pain or paræsthesia, healing of ulceration. In two of the cases recorded with "failure to halt progression of gangrene", amputation was considered imperative before sympathectomy was advised; the latter operation was advised to aid healing of the stump, which eventually occurred satisfactorily.

The findings in this series are in accord with those of other workers in regard to the beneficial effect of lumbar sympathectomy on the "distal" symptoms of occlusive arterial disease of the lower limbs.

"Intermediate-Term" Results

It is difficult to assess the long-term results of sympathectomy in occlusive arterial disease, as the early improvement may be offset later by extension of the pathological process in the limbs, or the patient may die from similar disease in the heart or brain. The expression "intermediate-term" has been used for the period from a few weeks to a few years after operation. Our assessment of the effects during this period is based on a study of 20 patients who were followed by an interview or letter (three patients) and whose operation had been performed more than three months previously. All these had some "distal" symptoms and 13 had claudication. The patient's assessment as to the effect of treatment is shown briefly in Table III.

"Distal" Symptoms

The patients who claimed that they had received no benefit were a man with gross bilateral atherosclerosis who had presented with gangrene of a foot later requiring amputation, two patients with long-standing thrombosis of the lower end of the abdominal aorta, and a diabetic patient with bilateral atherosclerosis and severe rest pain who had extension of his arterial occlusion some six weeks after operation. In the other 16 patients the early symptomatic relief had been maintained. In general, the symptomatic relief accorded well with the healthy condition of the feet on examination. After unilateral operation, the foot was often warmer on the sympathectomized side.

TABLE III

"Intermediate-term" Effect: Patient's Assessment

Effect	Distal Symptoms	Claudication
Benefit	16	6
No benefit	4	7
Not assessable	—	1[1]
Total	20	14

[1] Continuous rest pain.

Claudication

The effect of the operation on claudication was not so pronounced as on distal symptoms. In only three of the six patients who claimed to have received benefit did this seem clinically significant. It is difficult to assess the reliability of the patient's statement concerning his walking distance.

One patient stated that his walking distance had improved and was now 370 yards. According to his preoperative records his walking distance then was 500 to 600 yards.

One patient in his written reply stated that his walking distance had increased and "walking very slowly can go a mile without great discomfort". Close questioning at interview revealed that if he walked at the same rate as before operation his walking distance was unaltered.

Because of doubt concerning the patient's reliability in estimating his walking distance, we have attempted to judge this by a more objective test. The patient has been asked to walk at a constant rate over standard steps, and the number of circuits required for production of pain and the disappearance time have been noted. The results of step tests recorded before operation and during the period one to six months after operation have been compared. (These times have been chosen arbitrarily.

TABLE IV

Results of Step Tests Before and After Sympathectomy

Subject	Circuits (Steps: Mean and Range)		Mean Time per Circuit (Seconds)		Mean Disappearance Time (Seconds)		Patient's Assessment of Effect on Walking Distance
	Pre-operative	Post-operative	Pre-operative	Post-operative	Pre-operative	Post-operative	
L.G.	13 (6–19)	18 (10–26)	10	7	85	80	Increased
G.C.	55 (42–65)	37 (30–52)	10	8	135	130	No change
R.C.	13 (10–18)	9 (8–12)	8	10	85	110	—
W.L.	18 (12–24)	16 (14–18)	8	7·5	100	115	No change
A.McL.	16 (12–19)	17 (14–21)	8	7	90	110	Increased
F.P.	18 (15–24)	14 (9–18)	6	6	105	95	No change
G.L.	10 (7–13)	11 (7–13)	8·5	8·5	75	90	No change

It is considered that the walking distance immediately after operation may be influenced by factors other than sympathetic nervous interruption, and after several months it may have altered from the natural history of the disease.) Results of step tests in seven cases are shown in Table IV. (Six of these patients are included in the 14 patients with claudication in Table III. The other patient, G.C., had died.)

Unfortunately, insufficient observations have been made for proper statistical treatment in individual cases. A 25% improvement in the mean number of steps has been taken as a criterion of significant clinical improvement. On this basis, the condition of only one of the seven patients was improved.

We have no convincing evidence that sympathectomy is of value in intermittent claudication, but we cannot deny that it may help sometimes. There is also no evidence that claudication is made worse by sympathectomy. The operation may therefore be advised for patients with distal ischæmia and claudication, although the patient should be told that it is unlikely that his claudication will be much relieved.

DISCUSSION

The age of the patient did not appear to have any influence on the result, relief of distal ischæmia being obtained just as readily in the elderly as in young patients. The oldest patient in this series was aged eighty-five years. He obtained relief of rest pain and the colour of his foot improved.

Most of the patients in this series were men; but the women obtained relief just as readily.

Atherosclerosis was the most common condition represented in this series. Benefit was also obtained in the two cases of thromboangiitis.

The two patients with thrombosis of the aorta had some temporary improvement only, but in general the results were disappointing, and when questioned several months later they did not consider they had received benefit. In two of the patients with progression of gangrene, the femoral artery was blocked; but in these the ischæmic features were so severe that amputation was considered inevitable before operation.

Apart from these, the site of the block did not seem to have any great influence on the results, and improvement occurred in patients with occlusion of a large vessel such as the femoral or the smaller arteries of the leg or foot.

In most of the patients in this series symptoms had been present for several months. Good results were obtained in the three cases of acute arterial occlusion.

SUMMARY AND CONCLUSIONS

The immediate effect on the foot (warming, improved colour, decrease in rest pain) was usually good, definite improvement being observed in 21 of 28 patients.

The "intermediate-term" effect on distal ischæmic symptoms was also usually good, benefit being claimed by 16 out of 20 patients questioned three months to four years after operation.

Little relief occurred in intermittent claudication. Six of 14 patients with this symptom stated that they had received benefit. However, observations based on a step test before and after operation showed improvement in only one of seven patients.

Age, sex, pathological lesion, site of block (except possibly of occlusion of the lower end of the aorta, or of the iliac and upper part of femoral vessels) and chronicity of the lesion did not greatly influence the expected results.

In general, the results in this series agree with those of other workers in respect to the relief of " distal " symptoms (due to ischæmia of skin and nervous tissues). However, they are at variance with the findings of many observers who have stated that sympathectomy has relieved numerous patients with claudication. It is considered that the difference in findings in this respect may possibly be attributed to undue reliance placed by some investigators on the patient's assessment of his walking distance rather than on the results of more objective tests.

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STAPHYLOCOCCAL PNEUMONIA OCCURRING DURING AN INFLUENZA EPIDEMIC[1]

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DURING the months of August and September, 1953, cases of upper respiratory tract infection in Perth became more prevalent; it was evident that the disease had assumed epidemic proportions, and both the numbers affected and the severity of the illness were reflected in the temporary depletion of staff in hospitals, offices and similar organizations. Apart from its nuisance value to the community in this manner, little notice was taken of the epidemic until it was sharply punctuated by three deaths from acute staphylococcal pneumonia. A fourth patient suffered a similar but less severe infection and survived. These cases are thought worthy of recording because of the relationship between fulminating staphylococcal pneumonia and influenza occurring in young adults.

REPORTS OF CASES

CASE I.—A hospital nurse, aged nineteen years, reported that she had had sore eyes and a sore throat for twenty-four hours. The cough was non-productive and she had a raw feeling behind the sternum. Her temperature was 100° F., her pulse rate was 100 per minute, and the respirations numbered 20 per minute. The conjunctivæ were injected and the throat was moderately inflamed with some lymphoid enlargement. No abnormal physical signs were present in the chest. It was noted that the patient was menstruating. At this stage there seemed to be no reason to think that she had anything but a respiratory infection involving the upper and part at least of the lower respiratory tract. She was admitted to hospital and treated with aspirin and a cough mixture. The white blood cells numbered 12,000 per cubic millimetre, 90% being neutrophile cells showing toxic granulation.

Eighteen hours after her admission to hospital she became restless and slightly irrational, and presented a colour which was both flushed and slightly cyanosed. Her temperature was 101° F., and her pulse rate 120 per minute, and her respirations numbered 40 per minute. An occasional râle in the right axilla was discovered on examination of the chest, and although the X-ray appearances in the chest were within normal limits, it was only too clear at this stage that pneumonia was present. The oral administration of "Sulphamezathine" was begun, three grammes being given initially, followed by two grammes every four hours.

Three hours later, delirium, dyspnœa and cyanosis had increased; 200,000 units of penicillin were administered intramuscularly every three hours in addition to the "Sulphamezathine" previously administered. Shortly afterwards a small amount of darkly blood-stained purulent sputum was expectorated. Because of the continued deterioration in the patient's condition, one gramme of "Terramycin" was given orally and followed by 500 milligrammes every six hours. Oxygen was given continuously by intranasal catheter.

Twenty-seven hours after her admission to hospital the patient was gravely ill. Peripheral failure was of such a degree that the blood pressure could not be recorded; consequently it was decided to add not only erythromycin (one gramme by mouth) but also cortisone (200 milligrammes given intramuscularly).

Twenty-nine hours after her admission to hospital the patient died.

The post-mortem examination showed the subject to be a well-nourished girl, deeply cyanosed, and with a little blood-stained fluid coming from her mouth. Apart from a soft spleen of normal size, the abnormal findings were confined to the respiratory tract and the suprarenal glands. The mucosa of the trachea and bronchi was intensely hæmorrhagic. Both lower lobes of the lungs were entirely airless and plum-coloured; the upper lobes were less severely but similarly affected in a lobular distribution. Deeply blood-stained fluid dripped from the cut surface of the lung: there was no evidence of pus, nor were there any areas of necrosis or abscess formation.

Staphylococcus aureus, hæmolytic and coagulase-positive, was recovered from the throat swab and the sputum during life, as well as from lung tissue at the post-mortem examination (see Table I).

Microscopic examination showed that the bronchial mucosa was desquamated in several areas and in other areas there was a pseudo-membrane. The bronchi were filled with red cells, lymphocytes and a few epithelial cells, and there was peribronchial lymphocytic infiltration.

The cortex of the suprarenal glands was congested in its outer layers, and some small areas showed degeneration. The medulla was also congested, but there were no definite hæmorrhages.

Comment.—A case of fulminating staphylococcal pneumonia in a girl, aged nineteen years, is described; the condition followed an upper respiratory infection resembling influenza. A variety of therapy had no influence on the rapid progression of the disease.

[1] Received on July 2, 1954.

TABLE I

Sensitivity Tests on Staphylococci Recovered in Three Cases

Case Number: Source of Organism	Penicillin	Strepto-mycin	"Aureo-mycin"	"Chloro-mycetin"	"Terra-mycin"	Sulpha-diazine	Erythro-mycin
Case I[1]:							
Naso-pharynx ..	−	++	+	+	+		
Sputum	−	++	+	+	+		
Case II:							
Naso-pharynx ..	−	−	+	+	++	−	+
Blood	−	−	+	+	++	−	+
Lung *post mortem* ..	−	−	+	+	++	−	+
Case III:							
Spleen *post mortem* ..	++	+	−	−	+	−	+

[1] The organism was recovered from the lung *post mortem*, but sensitivity tests were not carried out.

CASE II.—A well-built boy, aged sixteen years, had his first symptoms two days before his admission to hospital. The initial symptoms were sore throat and headache, and after these his parents noticed a rash behind the ears, which, however, soon disappeared. Twenty-four hours after the onset the presence of fever and dyspnœa became obvious and he began to vomit. Early on the day of admission to hospital he became restless; within hours this restlessness progressed to delirium, and he became incontinent of both urine and fæces.

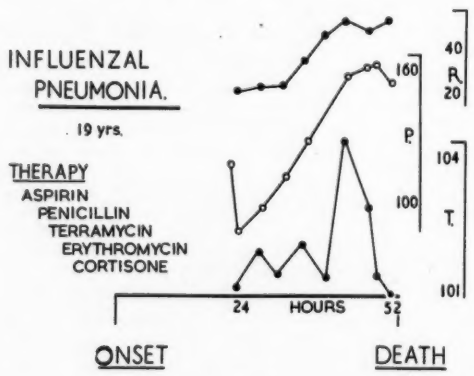

FIGURE I

Case I: The clinical course in a fatal case of staphylococcal influenzal pneumonia.

When admitted to hospital he lay curled up in bed on his side between periods of restless activity. His colour was a mixture of both pallor and cyanosis. The respirations were shallow and rapid (36 per minute); the pulse was poor in volume and regular in rhythm, and the rate was 130 per minute. The temperature was 104·2° F. The blood pressure was 130 millimetres of mercury, systolic, and 80 millimetres, diastolic. In addition to the rapid weak movements of the chest, air entry was diminished at the bases of both lungs, and an increase in pitch together with a prolonged expiratory phase was evident in numerous areas. There was some resistance to flexion of the cervical part of the spine in addition to a similar resistance to flexion of the hip with the lower limb straightened. The left ankle jerk could not be elicited.

Penicillin (1,000,000 units every four hours), "Sulphamezathine" (three grammes given intravenously, followed by two grammes given orally every four hours), and streptomycin (one gramme twice a day) were administered, and intragastric feeding was commenced. Paraldehyde (five to 10 cubic centimetres into a muscle) was given as a sedative.

The appearance of small purpuric spots on the buttocks, together with further signs of consolidation of the right lung, were evidence of the increasing severity of both septicæmia and pneumonia. Continued deterioration in the patient's general condition led to his death fourteen hours after his admission to hospital.

A post-mortem examination was performed seven hours after his death. Macroscopically evident abnormality was confined to the respiratory organs, apart from the few petechial spots on the buttocks.

The mucosa of the trachea and bronchi was grossly hæmorrhagic. The lungs, which did not collapse when the thorax was opened, were a pale pink colour with large dark red areas, often wedge-shaped, the base of these areas being situated on the pleural surface, which was covered by a fine layer of fibrin. Within the areas of discoloration—and close to their pleural surface—were small irregular areas of necrosis, mostly only a few millimetres in diameter, but tending to coalesce to form the precursor of an abscess. Pus was not evident. Deeply blood-stained fluid dripped from the cut surface of the affected areas. The lower lobe of the right lung was the most severely affected.

Microscopic examination showed that the tracheal mucosa had been completely destroyed and replaced by a dense network containing a few polymorphonuclear cells, but mainly large mononuclear cells. In the submucosal glands central necrosis was evident. Alveolar tissue was extensively destroyed, the exudate being grossly hæmorrhagic; here and there were small accumulations of mononuclear cells and relatively few polymorphonuclear cells; within these collections were groups of Gram-positive cocci. There was fragmentation of cardiac muscle fibres; cross-striation was broken up; interstitial œdema was present, while the intercalated disks were greatly swollen.

Bacteriological investigation was carried out on a nasopharyngeal swab and on blood taken from the patient soon after his admission to hospital, in addition to secretions from the lung obtained at the post-mortem examination. *Staphylococcus aureus*, hæmolitic and coagulase-positive, was recovered from each specimen. Tests for sensitivity by the disk method gave the results shown in Table I.

Comment. — This case of staphylococcal pneumonia and septicæmia in a boy, aged sixteen years, resembles the previous one in its rapid progression following an upper respiratory tract infection.

CASE III.—A young married woman, aged nineteen years, had complained of a productive cough and general malaise for five days prior to her admission to hospital. Her husband stated that other members of the household had recently had " 'flu ". Sixteen hours before her admission her condition became rapidly worse: she became stuporose and diarrhœa occurred.

When admitted to hospital the patient was irrational, with rapid, shallow respirations (34 per minute). Her colour was flushed and the conjunctivæ were suffused. The pulse was regular in rhythm, of poor volume and rapid rate (92 per minute). The blood pressure was 80 millimetres of mercury, systolic, and 60 millimetres, diastolic. The tongue was dry and furred and her breath had a sweet odour. At the base of the right lung the percussion note was impaired, breath sounds were weaker than on the left, and expiration was prolonged with a rise in pitch.

The administration of penicillin (200,000 units every four hours) and " Sulphatriad " (two grammes *statim*, one gramme every four hours) was commenced, but the patient became rapidly worse; her temperature rose to 105·4° F., and she died ten hours after her admission to hospital.

A post-mortem examination was carried out eight hours after death. The body was well developed, though rather thin. Cyanotic blotches were present on the shoulders and face. Apart from slight congestion, no lesion was apparent in the left lung; the right lung was heavy with a faint layer of fibrin covering the lower lobe and part of the upper lobe; the whole of the lower lobe was dark red in colour, and when it was cut, the appearance resembled that of red hepatization. Within this area were smaller wedge-shaped sections; some of these areas contained collections of small abscesses (two to three millimetres in diameter) filled with pale yellow pus.

The mucosa of the trachea was covered by a fine layer of mucopus; it was very hæmorrhagic. Coagulase-positive staphylococci were grown on culture from the spleen, which was soft and pale red (Table I).

Microscopic examination of the trachea revealed almost complete destruction of the mucosa; submucosal glands were still present, and vessels in this area showed intense hyperæmia. The fibrinous exudate covering the mucosa contained a few polymorphonuclear cells and leucocytes. The alveoli were destroyed and replaced by an extensive hæmorrhagic exudate. Small abscesses were seen containing polymorphonuclear leucocytes and mononuclear cells, together with clusters of Gram-positive cocci.

In the heart muscle similar changes to those in Case II were seen.

Comment. — After an infection clinically resembling influenza and similar to infections recently suffered by her family, a previously healthy young woman rapidly succumbed to staphylococcal pneumonia and septicæmia.

DISCUSSION

There can be no doubt that these three patients suffered from staphylococcal pneumonia.

The evidence that they were infected by the influenzal virus as well as the staphylococcus is indirect, though overwhelmingly in favour of this hypothesis. The early signs and symptoms in each case were in no way pathognomonic, but were at least consistent with influenza. Secondly, these cases occurred during the height of an epidemic which was proved to be due to influenza B virus. In this we had the assistance of Mr. J. White, Dr. S. G. Anderson and Dr. D. M. McLean (whose results are published elsewhere—1954), who examined a small number of throat washings and paired sera taken from hospital nurses whose illness clinically resembled influenza. The virus was not isolated from any of the throat washings; but in six patients pairs of sera (with 10 to 14 days between each specimen) were examined and three were found to have developed antibody to influenza B soon after the first specimen of serum was taken; while one (Case 4) had recently recovered from a similar infection.

TABLE II

Reciprocal of Titres of Complement-Fixing Antibody Against Influenza Virus B in Four Cases

Case Number	First Serum Specimen	Second Serum Specimen
I	5	30
II	5	60
III	5	15
IV	30	30

Confirmation of these findings was obtained a little later when the Public Health Laboratories collected sera from other parts of the community in and around Perth (Snow, 1953). These specimens were examined by the Commonwealth Serum Laboratories, where a rise in antibodies for influenza B virus was found.

In addition to the clinical findings, and the fact that influenza was rife in the community at the time, there are two other considerations which strengthen the hypothesis that these patients suffered from influenzal staphylococcal pneumonia. The first is that ever since the influenza pandemic of 1918–1919, when the death rate in the twenty to forty years age group was so high, the phenomenon of young healthy adults succumbing rapidly to staphylococcal pneumonia has strongly suggested the combined effect of the influenza virus and the staphylococcus. It is worthy of note that whereas in the 1918–1919 pandemic bacteria isolated from these cases were a heterogenous collection, in later epidemics the staphylococcus has invariably been found to be the bacterium responsible for the disease in its fulminating form (Stuart-Harris, 1953). The second is the macroscopically and microscopically evident abnormality in the trachea and main bronchi.

One would hesitate to suppose that the necrotizing process causing complete destruction of the ciliated columnar epithelium in the trachea is pathognomonic of influenzal infection in man. A similar cytological picture is seen in animal experiments with influenza virus (Stuart-Harris, 1953). The only chance of studying the effect of the virus on the human respiratory tract has been when the virus is associated with bacteria producing fulminating and fatal pneumonia. Winternitz, as long ago as 1920, described the histological findings, presenting a picture clearly distinct from that seen in the common types of bacterial pneumonia. Four years after the first isolation of the influenza virus, Scadding (1937) recorded three cases in which pulmonary lesions resulted from a combination of virus and staphylococcus. A similar report was made by Himmelweit (1943). Mulder and Verdonk (1949) described the early epithelial changes in fulminating staphylococcal pneumonia; they gave good reasons for assuming that the histological appearances in their case represent changes effected by the influenza virus as well as by the staphylococcus. Yet one must agree with Stuart-Harris (1953) that "no one has convincingly demonstrated that influenza virus unaccompanied by bacterial superinfection will produce a necrotic epithelial reaction in the trachea and bronchioles in man". The histological evidence in the present cases is nevertheless strongly suggestive, even if not entirely convincing. Destruction of the trachea and main bronchi was seen to a gross degree, particularly in Cases II and III. These lesions, suggesting the action of a virus, are in contrast to the macroscopic and microscopic evidence of bacterial inflammation—in places approaching abscess formation—found in the region of the alveoli.

We believe that the foregoing evidence confirms the hypothesis that our three patients suffered from an illness caused by both virus and bacteria; there is, however, little information to show in what manner and under what circumstances the influenza virus and the staphylococcus form a combination which is lethal to the healthy young adult.

The age of these patients reminds us that the greatest mortality in the 1919 epidemic was in the twenty to forty years age group, so that it is unlikely that poor resistance on the part of the host is responsible for the severity of the infection. If this was so, one would expect to find the greater mortality in the elderly with their background of chronic bronchitis and cardiac decompensation. However, a distinction should be made between the resistance of the host as a whole and the possibility of an altered state of one part of the host—his respiratory tract. Investigations of importance related to the role of mucus in respiratory infection have been undertaken in recent years with regard to both virus and bacteria. Francis (1940) has shown that naso-pharyngeal secretions possess the ability to inhibit the action of influenza virus. More recently in Australia Fazekas de St. Groth (1951) has added to this knowledge; and it is possible that the difference between the benign course of influenza and the fulminating march of influenzal staphylococcal pneumonia is in some way related to the anti-viral properties of a respiratory mucin.

The virulence of the staphylococcus and its insensitivity to penicillin are often cited as the main contributing factors. However, Mulder (1952) having observed 18 cases, found only one in which the organism was insensitive to penicillin. Although in one of the present cases the organism was sensitive to the drug, the frequent occurrence of the penicillin-insensitive staphylococcus today must be viewed with grave concern. There is good reason to believe that this organism will behave towards erythromycin as it has towards other antibiotics which have been used freely and often indiscriminately. If this is so, the chances of combating the disease when it has reached its fulminating form—slender as they are even with early recognition—will dwindle to negligible proportions.

The extent of the contribution of the virus to this form of influenzal pneumonia is still unknown. There is no evidence that viræmia occurs, or that its effect spreads more widely than the respiratory tract. Within this field there are two possibilities: first, that by destroying the local means of defence, the virus allows the staphylococcus to extend its invasion beyond the respiratory system and cause fatal septicæmia; secondly, that mutation, or a form of hybridization between related viruses as has been observed in the laboratory (Burnet, 1953), may be responsible for the appearance of a particularly virulent strain, or may confer on the virus the ability to leave its usual confines of the bronchial tree and penetrate into the parenchyma of the lung (Mulder and Stuart-Harris, 1953).

It is not possible, at this stage of our understanding, to assess accurately the respective roles of virus and bacteria in this fatal disease. In the absence of specific antiviral therapy everything must be brought to bear against the staphylococcus. When, in a case of typical benign upper respiratory infection, there are

signs of a rapid deterioration in the patient's condition, such as an increase in respiratory rate and pulse rate with evidence of impending circulatory failure and lung involvement, it is reasonable to assume that this disease complex is developing, and imperative that large doses of one of the broad-spectrum antibiotics be employed. The danger of the staphylococcus becoming insensitive to erythromycin has already been mentioned; but this would seem to be an occasion when the use of this drug is justified without waiting for proof that the organism is insensitive to penicillin. Staphylococcal antitoxin would seem to be of little value. The use of cortisone during the stage of peripheral circulatory failure must be considered. Hæmorrhage of the adrenal glands reported by Mulder (1952) adds weight to the argument for the use of this drug.

SUMMARY AND CONCLUSIONS

An epidemic caused by the influenza B virus occurred in Perth in the spring of 1953.

Three cases of fatal staphylococcal pneumonia in previously healthy young adults are reported. The problem of relating these three cases to the influenza epidemic is considered. On the basis of clinical, serological and pathological evidence we believe that the present cases belong to the category of "influenzal staphylococcal pneumonia".

The occurrence of this form of pneumonia in healthy young adults presents problems in ætiology which are as yet unanswered. The possible roles of virus and bacteria are discussed.

While further elucidation of the pathogenesis of influenzal staphylococcal pneumonia is awaited, our efforts in treatment must be directed as early as possible against the staphylococcus. The slight chance of success is a reflection both of the fulminating nature of this disease and of the alarming situation which has grown out of the success of the staphylococcus in its survival of the antibiotic onslaught.

ACKNOWLEDGEMENTS

We wish to acknowledge our gratitude to Dr. I. O. Thorburn for permission to publish details of Case II; to Dr. T. R. Lubbe and Dr. V. Fergusson Stewart for details of post-mortem findings; and to Mr. A. F. Drummond, of the Public Health Laboratories, for his help in the collection and dispatch of serum specimens. Proof of the existence of an influenza epidemic was the result of investigations carried out by Dr. S. G. Anderson and Dr. D. McLean, of the Walter and Eliza Hall Institute of Medical Research, Melbourne, and Mr. J. White of the Fairfield Hospital, Melbourne, whose assistance we also wish to acknowledge.

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ADDENDUM

We have recently had the opportunity of observing another case of this type of staphylococcal pneumonia; this case occurred in the following winter, during an epidemic of what appeared clinically to be influenza. The clinical history is as follows.

A boy, aged nine years, under the care of Dr. Walter Seed, and convalescent from rheumatic fever, after four weeks in hospital, developed a temperature of 102·6° F. The cause was not obvious. There was a discharging furuncle of the lower limb, and no clinical or radiographic evidence of a chest lesion. The leucocyte count was 7600 per cubic millimetre. After twenty-four hours the boy's temperature had risen to 105·2° F., and it became obvious that he was suffering from a severe form of pneumonia.

He was extremely pale, but with cyanotic lips, a pulse rate of 130 per minute and a respiratory rate of 30 per minute; his voice was hoarse, and adventitiæ were to be heard at the bases of both lungs. On the third day he remained critically ill, the respiratory rate

rising to 40 per minute, with retraction of the lower ribs anteriorly on each shortened inspiration. X-ray examination of his chest now revealed a patchy loss of translucency in both lung fields.

He had been having penicillin by mouth (100,000 units three times daily). When his pyrexia began, intramuscular injections of the drug (250,000 units every six hours on the second day) were substituted, and "Sulphamezathine" (one gramme every four hours) was added. On the third day, when it was realized that this acute and severe condition was most probably influenzal staphylococcal pneumonia, streptomycin (one gramme, followed by 0·5 gramme eight hourly), erythromycin (0·5 gramme four hourly), and "Terramycin" (125 milligrammes intravenously every eight hours) were substituted for the former antibacterial drugs. His condition remained critical for several days, but by the end of a week there were encouraging signs of recovery. During this latter period partial collapse of the lower lobe of the left lung developed, but this gradually responded to postural drainage and breathing exercises. He was discharged from hospital four weeks after the onset of his pneumonia, the X-ray films of the chest showing no more than a small opacity at the base of the left lung.

Examination of paired sera showed a sharp rise in antibody titre to influenza virus Type A. *Staphylococcus aureus*, insensitive to penicillin, was cultured from the furuncle on the leg, and was also obtained from a sub-culture of the blood. A specimen of sputum was unfortunately unobtainable before the administration of antibacterial drugs was commenced. This evidence considerably strengthens the clinical impression that this fulminating form of pneumonia following closely on the symptoms of influenza, in the presence of a proven staphylococcal focus, was in fact influenzal staphylococcal pneumonia. The presence of the patient in hospital at the onset, which made possible prompt recognition and treatment, undoubtedly played a part in his recovery; nevertheless, it must be admitted that the hospital was probably the source both of the virus and of the bacterium.

GASTRIC AND INTESTINAL MOTILITY STUDIES WITH MORPHINE, ATROPINE, HEXAMETHONIUM BROMIDE AND " BANTHINE "[1]

RODERICK ANDREW[2]

From the Baker Medical Research Institute and Clinical Research Unit, Alfred Hospital, Melbourne

THE effects of morphine, atropine, hexamethonium bromide and " Banthine " on gastro-intestinal motility have been observed in the following investigations by the use of tandem air-filled balloons. These are placed in the gastric antrum, the duodenal cap and the upper part of the jejunum, and motility under the influence of these drugs has been studied in these regions.

Over many years balloon techniques have provided the foundation for much of our knowledge of the effect of drugs on gastro-intestinal motility. There are limitations in the accuracy of these methods, and for quantitative work, when pressures are measured, an electrical transducer is necessary (Quigley and Brody, 1952). But the original and simple method of recording, by the use of balloons, gives results of unquestioned validity (Hightower, 1952) when qualitative changes are being studied. The standard balloon apparatus has the disadvantage of its size and the discomfort which it causes ; but for reasons given in other studies (Andrew, 1954) these factors have little effect on the conclusions drawn from the tracings produced.

Certain facts about normal motility, and the care needed in interpreting drug action, need emphasis before the results are analysed. In the gastric antrum large waves (Type I), which are of a peristaltic nature, may occur either isolated or in a rhythmic series lasting for a few minutes up to about one hour. Small waves (Type II) may occur as a rhythmic series or mixed in with large waves. After a burst of activity periods of quiescence follow, of a few minutes' duration, or lasting from thirty to sixty minutes. Tetany (Type III), consisting of small waves at a rate of nine to 12 per minute superimposed on a spasm which raises the base-line, while uncommon in the stomach, is the usual form of activity in duodenum and jejunum, although small waves are also frequently seen in these sites. Tetany may be sustained for many minutes, or may occur as rhythmic or non-rhythmic tetany waves lasting from thirty seconds to one and a half minutes. Spontaneous changes in frequency often occur in the space of a few minutes. However, quiescence of all waves is very uncommon in the duodenum and jejunum for more than a few minutes. Finally, the waves in the three sites observed may occur in an harmonious manner, with waves passing from antrum to duodenal cap and thence to the jejunum, or the motility pattern may be completely autonomous, with each contiguous segment of gut functioning independently both in timing and in type of wave movement (Andrew, 1954).

Therefore, observations of drug effects must be interpreted with some caution, due regard being paid to the complexity of wave form and to the speed with which one form of motor activity may change spontaneously to one quite different. It follows that " acute " experiments, in which the drug is given by injection and an effect quickly produced, are less liable to interpretative error than those experiments in which the drug is given orally and time of absorption becomes an important factor.

Clearly, then, an understanding of motor physiology is basic in pharmacological interpretation. In addition to the type of gut activity at the moment of drug administration, other factors will play their part in the individual's variation in response: the equanimity or otherwise of the subject, the recording methods, the size of dose, and the route by which it is given being all concerned.

METHODS

The apparatus used in these studies has already been described in an investigation of gastro-intestinal motility in normal subjects (Andrew, 1954). Briefly, it consists of three tandem air-filled balloons attached to a four-lumen Miller-Abbott tube, which is swallowed by the patient. The position of the balloons

[1] Received on June 15, 1954.

[2] Part of this work has been done during the tenure of the Sol Green Research Scholarship.

is adjusted under radiological screen control so that the balloons lie (i) in the pyloric antrum, (ii) in the duodenal cap, (iii) in the most proximal portion of the jejunum (Figure I). The fourth channel is for the injection of drugs in solution. Each channel is connected to a water manometer, and motility is recorded on a kymograph. After a preliminary observation period of about thirty minutes, the drug is either injected parenterally or run through the fourth channel of the Miller-Abbott tube.

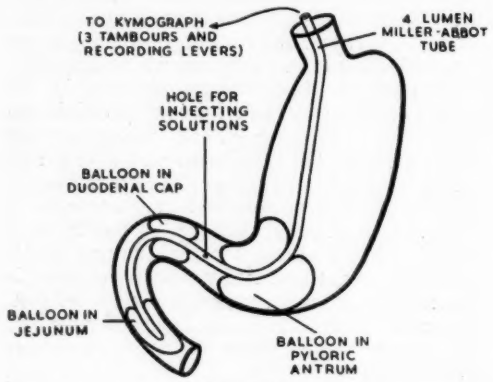

FIGURE I

Diagram of four-lumen Miller-Abbott tube with balloons in position

SUBJECTS

Twelve subjects were used for the investigations. Of these, eight had active duodenal ulceration and one an active gastric ulcer, and three were normal students.

The choice of subjects was mainly dictated by the difficulties of obtaining patients who agreed to the test and were able to cooperate. Conclusions drawn from the effect of drugs on normal subjects and those with diseased stomach or duodenum might be difficult to analyse if it was known that their motility patterns varied in any characteristic way. Considerable changes in the recording pattern have been shown to occur in an unpredictable way in a normal individual over the one to two hours' observation (Andrew, 1954). Analysis of the present records and of many others (unpublished data) have not shown any features in the type of waves, in their rate, speed, or integration, or in the frequency of quiescent periods, which distinguish normal subjects from those with active duodenal or gastric ulceration. A statistical analysis by Foulk *et alii* (1954) of duodenal and upper jejunal motility patterns has been based on over 20,000 measurements. They have shown in normal subjects and in patients with duodenal ulceration that there is no statistical difference for small (Type I) or tetany (Type III) waves in rate, in percentage time of activity, or in percentage time of non-rhythmic and rhythmic waves.

The measurable changes which drugs can induce in the motility pattern of the gut are either (i) the induction of relative or absolute quiescence by the inhibition of waves, or (ii) augmentation of waves or the production of tetany. For reasons stated above, the basic problem of motility for normal subjects and for those with peptic ulceration can be regarded as the same. Whether the response to different drugs or to different doses of the same drug is influenced by a pathological state in humans is not clear from the following experiments, or from any other published work. There is such a wide range of basic response with varying doses in individuals that the problem would be exceedingly difficult to answer in a satisfactory manner.

RESULTS

A preliminary study with 200 millilitres of normal saline run through the fourth channel of the Miller-Abbott tube (Figure I) was undertaken in order to see whether this would alter intraluminal pressure, or have an effect on the motility pattern. Figure II shows that

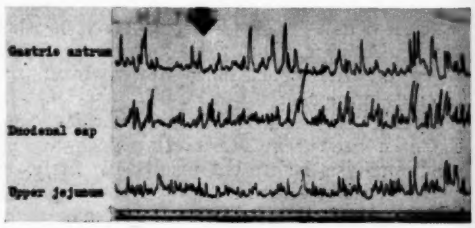

FIGURE II

Normal saline (200 millilitres) run in at arrow. No effect on motility. Time interval, two seconds

no such change resulted. " Banthine " was the only drug in these studies which was given orally (that is, injected down the tube), and in each case only 10 millilitres of water were used for its suspension.

Morphine

Two subjects were studied, and to each of them, morphine sulphate, 16 milligrammes (one-quarter of a grain) was given subcutaneously.

CASE I.—This was a case of active duodenal ulceration (Figure III). In the gastric antrum, inhibition occurred within two minutes and lasted for five minutes, and then normal activity returned for the sixty minutes of observation. In the duodenal cap, within two minutes tetany occurred lasting for twelve minutes. Small waves then followed for fifty-three minutes of observation. In the jejunum, within two minutes tetany occurred lasting for twenty-four minutes, and then small and large waves followed for forty-one minutes of observation.

CASE II.—This was a case of active duodenal ulcer (Figure IV). In the gastric antrum, tetany occurred within ten minutes and lasted for five minutes, and then there was quiescence for thirty-one minutes followed by small or large waves. In the duodenal cap, tetany occurred within two minutes and lasted for two and a half minutes; then tetany waves followed for six minutes, to be succeeded by a sustained tetany for twenty-four minutes, then tetany waves for the twenty-eight minutes of observation. In the jejunum, tetany occurred within two minutes and was sustained for thirty-two minutes, and was then followed by small and tetany waves for twenty-eight minutes of observation.

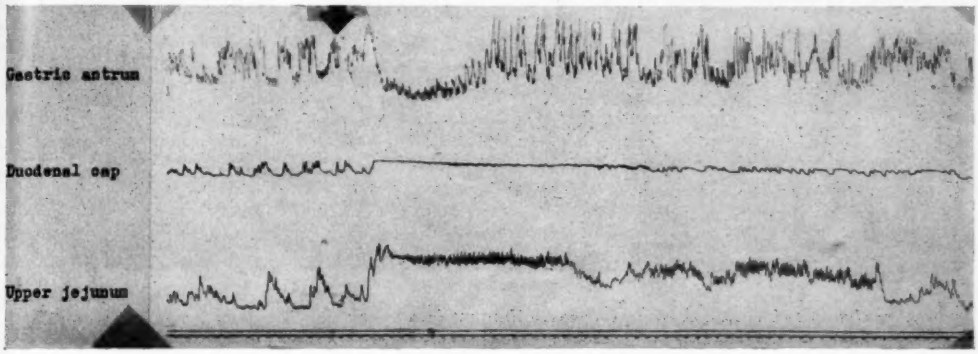

FIGURE III

Morphine (one-quarter grain) given subcutaneously at arrow. Time interval, thirty seconds

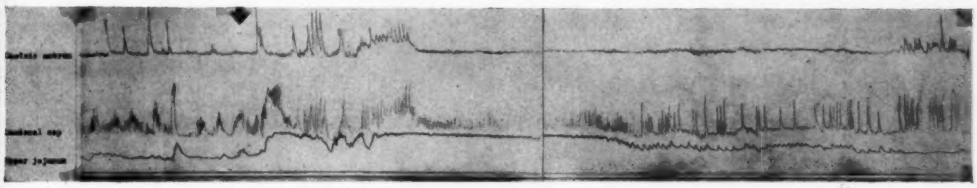

FIGURE IV

Morphine (one-quarter grain) given subcutaneously at arrow. Time interval, thirty seconds

Atropine

CASE III.—This was a case of active duodenal ulceration (Figure V). Atropine sulphate, 0·6 milligramme (one-hundredth grain) was given intravenously. In the gastric antrum, complete inhibition occurred in one minute, lasting for eight minutes, and was followed by small waves for twenty-nine minutes; then large waves returned (not shown in Figure V). In the duodenal cap, inhibition occurred in one minute for twenty minutes, and then small tetany waves returned. In the jejunum, inhibition occurred within four minutes and lasted for eighteen minutes, then small and tetany waves returned.

CASE IV.—This was a case of active gastric ulcer. Atropine sulphate, 0·6 milligramme (one-hundredth grain), was given subcutaneously. In the gastric antrum, inhibition occurred within one minute and lasted for the forty minutes of observation. In the duodenal cap, partial inhibition of tetany waves occurred within two minutes and lasted for forty minutes. A few low tetany waves at long intervals continued in this period of partial inhibition. In the jejunum, tetany occurred within eight minutes and lasted for ten minutes. Then partial inhibition followed for twenty-five minutes, infrequent low tetany waves continuing.

CASE V.—This was a case of active duodenal ulcer. Atropine sulphate, 0·3 milligramme (one-two-hundredth grain), was given intravenously. In the gastric antrum, no effect was observed. In the duodenal cap, inhibition of tetany waves for four and a half minutes occurred. In the jejunum, inhibition of tetany waves for eight minutes occurred.

CASE VI.—This subject was a normal control. Atropine sulphate, 0·3 milligramme (one-two-hundredth grain), was given subcutaneously. No effect

was observed on the gastric, duodenal or jejunal tracing in forty-five minutes; gastric large waves continued and duodenal and jejunal tetany and small waves continued.

Hexamethonium Bromide

CASE VII.—This was a case of active duodenal ulcer (Figure VI). Hexamethonium bromide, 100 milligrammes, was given intramuscularly. In the gastric antrum, inhibition occurred within one minute and lasted for eighty-five minutes of observation. In the duodenal cap, inhibition occurred within one minute and lasted for eighty-five minutes of observation. In the jejunum, inhibition occurred within one minute and lasted for eighty-five minutes of observation.

" Banthine "

CASE VIII.—This subject was a normal control (Figure VII). " Banthine ", 100 milligrammes in 10 millilitres of water, was given orally (introduced through tube which opens between the gastric and duodenal balloons). In the gastric antrum, inhibition occurred in five minutes (except for two large waves) and lasted for sixty-five minutes of observation. In the duodenal cap, inhibition occurred in five minutes and lasted for sixty-five minutes of observation. In the jejunum, inhibition occurred in five minutes and lasted for sixty-five minutes of observation.

CASE IX.—This subject was a normal control (Figure VIII). " Banthine ", 100 milligrammes in 10 millilitres of water, was given orally (as in Case

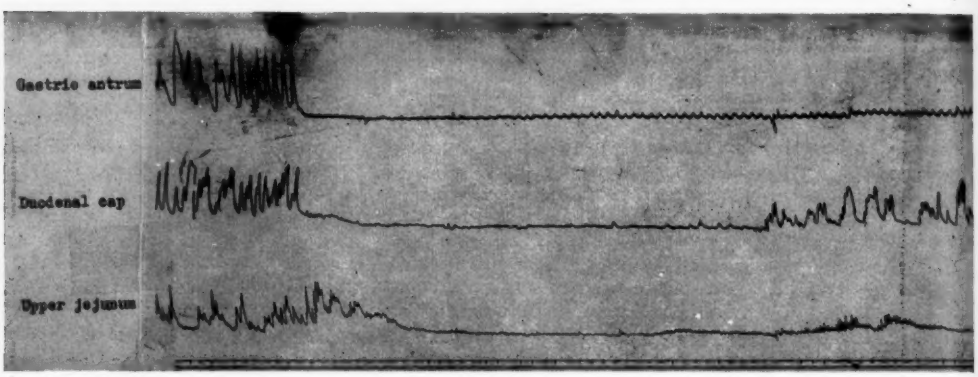

FIGURE V

Atropine (one-hundredth grain) given intravenously at arrow. Time interval, thirty seconds

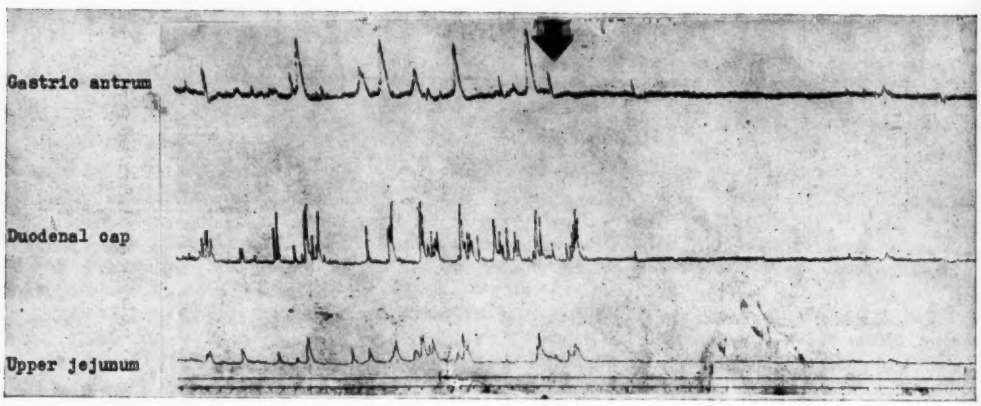

FIGURE VI

Hexamethonium bromide (100 milligrammes) given intramuscularly at arrow. Time interval, thirty seconds

VIII). In the gastric antrum, partial inhibition occurred in about thirty minutes; small and large waves continued at longer intervals. Pre-drug motility recurred in another forty minutes. In the duodenal cap, inhibition of tetany waves occurred after eighteen minutes. Small waves only were seen thereafter for fifty minutes of observation. In the jejunum, inhibition of tetany waves occurred within eighteen minutes. Small waves only were seen thereafter for fifty minutes of observation.

CASE X.—This subject was a normal control. "Banthine", 100 milligrammes in 10 millilitres of water were given orally as in Case VIII. In the gastric antrum, no change of motility occurred for seventy-two minutes of observation. In the duodenal cap, inhibition occurred within nine minutes of tetany waves and lasted for fifty-three minutes, small waves persisting; then tetany waves reappeared. In the jejunum, inhibition of tetany waves occurred within twelve minutes, small waves persisting for sixty minutes of observation.

CASE XI.—This was a case of active duodenal ulcer. "Banthine", 100 milligrammes in 10 millilitres of water, was given orally as in Case VIII. In the gastric antrum, complete inhibition occurred within twenty-six minutes and lasted for nineteen minutes of observation. In the duodenal cap, partial inhibition occurred within twenty-six minutes; small waves only were present for nineteen minutes of observation. In the jejunum, partial inhibition occurred within twenty-six minutes. Thereafter only small waves were present for nineteen minutes of observation.

CASE XII.—This was a case of active duodenal ulcer. "Banthine", 100 milligrammes in 10 millilitres of water, was given orally as in Case VIII. In the gastric antrum, complete inhibition occurred in nine minutes and lasted for sixty-five minutes of observation. In the duodenal cap, inhibition of tetany waves occurred within nine minutes, small waves only occurring for the sixty-five minutes of observation. In the jejunum, inhibition of tetany waves occurred within sixteen minutes, small waves persisting for the fifty-eight minutes of observation.

CASE XIII.—This was a case of active duodenal ulcer. "Banthine", 100 milligrammes in 10 millilitres of water, was given orally as in Case VIII. In the gastric antrum, no change occurred in motility for the sixty-one minutes of observation. In the duodenal cap, partial inhibition of tetany waves followed within six minutes, but these recurred thereafter at long intervals (three to five minutes) for the fifty-five minutes of observation. In the jejunum, partial inhibition of tetany waves followed within ten minutes, occurring thereafter at longer intervals (three to five minutes) for the fifty-one minutes of observation.

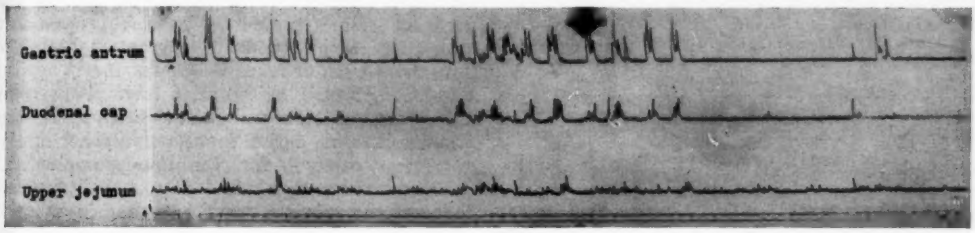

FIGURE VII

"Banthine" (100 milligrammes) given orally at arrow. Time interval, thirty seconds

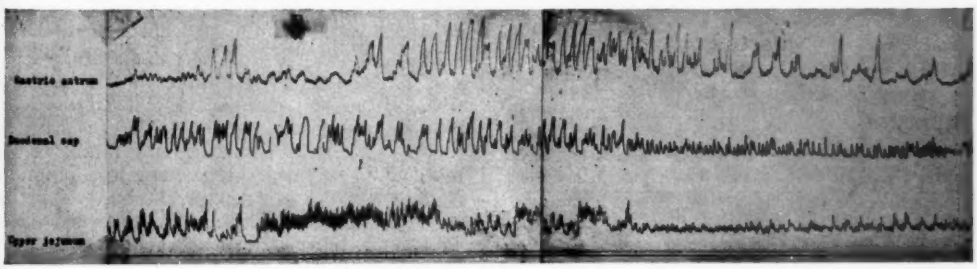

FIGURE VIII

"Banthine" (100 milligrammes) given orally at arrow. Time interval, thirty seconds

SUMMARY OF RESULTS

1. In the two cases in which the effect of morphine was tested, the main effect appeared to be spasmogenic on the duodenal cap and jejunum. This action was apparent within two minutes in each case.

2. Atropine was tested in four cases. With the larger dose (0·6 milligramme—one-hundredth of a grain—in two cases) given intravenously or subcutaneously, obvious striking spasmolytic effects were observed in the three sites. The reaction occurred within one minute in each case. With a smaller dose (0·3 milligramme—one two-hundredth of a grain—in two cases), either no effect or partial inhibition occurred.

3. Hexamethonium bromide was tried in one case. Complete inhibition of all waves in the three sites was apparent within one minute.

TABLE I

Time in Minutes at which Inhibition (Partial Inhibition, P) Occurred after 100 Milligrammes of "Banthine" given Orally. The minus sign indicates no change in motility

Site	Case Number					
	VIII	IX	X	XI	XII	XIII
Gastric antrum	5	30P	26	—	9	—
Duodenal cap	5	18P	26P	9P	9P	6P
Jejunum ..	5	18P	26P	12P	16P	10P

4. "Banthine" was tested in six cases. "Banthine" has an inhibitory effect on motility. Table I shows the time in minutes at which these effects were noted.

The duration in minutes of these effects is shown in Table II.

TABLE II

Duration in Minutes of Inhibition, Complete or Partial (see Table I). Plus sign indicates end of record

Site	Case Number					
	VIII	IX	X	XI	XII	XIII
Gastric antrum	65	40	19	—	65+	—
Duodenal cap	65	50	19	53	65+	55+
Jejunum ..	65	50	19	60+	58+	51+

From Tables I and II it can be seen that either partial or complete inhibition occurred in all six cases at the duodenal and jejunal sites, and in the stomach in four of the six patients. These effects occurred within five to thirty minutes of the administration, and were sustained for nineteen to sixty-five or more minutes.

DISCUSSION

In general these results confirm the work of many investigators using balloon techniques, which has been well summarized by Rowlands (1952) and by Code, Hightower and Morlock (1952).

With morphine, the effects carefully studied by Rowlands *et alii* (1950) were confirmed, the main feature being the resulting tetany of duodenum and small intestine following its administration. This is, no doubt, the cause of its constipating action through retardation of the passage of material in the intestine.

The clinical effect of atropine is notoriously variable in different patients. Generally in clinical medicine, the belladonna alkaloids are used for their spasmolytic effect. But the impressive experimental results are not matched by their clinical usefulness, which is limited for reasons not yet clear. The relatively short period of action, and the uncomfortable side-effects when adequate doses are used, are no doubt factors in this discrepancy. Pain from the gut, of many different origins, is the commonest indication for the exhibition of the atropine group — for example, œsophagitis, hiatus hernia, gastric and duodenal ulceration, ulcerative colitis, unstable colon and the "functional dyspepsias". But the mechanism of pain is still so obscure in these conditions that relative failure of relief, even by an effective spasmolytic agent in adequate doses, probably means that changes in gut tension are not always the sole source of painful impulses even when ulceration of the mucosa is absent.

Hexamethonium bromide was shown by Kay and Smith (1950) to inhibit gastric motility when given intramuscularly. This it does by blocking autonomic ganglia. The one case in the present study shows striking inhibition of motility in stomach, duodenum and jejunum (Figure VI). This probably accounts for the constipating effect so commonly observed when the drug is used for hypertension.

"Banthine", from the studies here presented, has an inhibitory effect on gastric and small bowel activity; this confirms the work of earlier observers (Longino *et alii*, 1950; Abbott *et alii*, 1952). However, there is considerable variation in the effects observed when 100 milligrammes are given orally; they range from complete sustained inhibition of all waves from the three recorded sites, to partial and transitory inhibition which involves duodenum and jejunum only. The effects observed are due to a blocking action both on the autonomic ganglia and on effector organs, but predominantly the inhibitory action is on the vagus. This drug has been shown to reduce the pain of active duodenal ulceration (Winkelstein, 1952; Friedlander, 1954). Clinical impressions made over three years by the

author have confirmed its usefulness. How this is accomplished is not certain. Not only is motility in the stomach and small bowel reduced or inhibited, but the amount of free acid is significantly reduced (Longino *et alii*, 1950; Andrew and Kelly, 1954). Unfortunately there is still no certainty of the cause of pain in peptic ulceration. There is considerable evidence that acid acting on the ulcer is an important factor, although there are contrary observations that motility disorders are more important. This being so, clinical interpretation of the physiological effects of "Banthine" here presented must await further knowledge of pain mechanism in peptic ulceration.

These results do not reveal in any clear-cut way whether the normal gut responds to the drugs used differently from the stomach or duodenum which is the seat of peptic ulceration, nor are they designed to do so. The indications from these data are that size of dose and individual susceptibility are probably of greater importance than the presence or absence of peptic ulceration.

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CEREBRAL SHRINKING[1]

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A VOLUMINOUS literature has accumulated upon the topic of cerebral swelling—a conception which was introduced as a reaction from the doctrine that all swollen brains are necessarily œdematous (Greenfield, 1939; Scheinker and Segerberg, 1948). The terms "cerebral œdema" and "cerebral swelling" have been used by some as synonyms, but this usage is quite unjustifiable, since factors other than œdema may cause increase in volume of the brain (Greenfield, 1942). The debate as to the frequency and pathogenesis of cerebral œdema continues, and the tendency to regard it as an inexplicable direct result of the mere presence of a tumour or other lesion is still seen. Further, the argument is still frequently advanced that œdema raises the intracranial pressure, causing cerebral compression, which may result in fatal ischæmia.

Cerebral shrinking, on the other hand, has been almost ignored, yet it is at least as common and as important as swelling. Perhaps it is ignored because it is not a cause of cerebral compression, which has achieved a quite undeserved monopoly of our interest in cases of space-occupying lesion. Yet a study of the factors involved in reducing brain volume casts an interesting light not only upon the converse state of cerebral swelling, but also upon the course of many intracranial lesions.

Cerebral swelling is defined as increase of the brain volume beyond the normal 92% to 96% of the cranial capacity, not due to localized space-occupying lesion or to excess of free fluid. The former proviso excludes tumour, abscess, hæmatoma *et cetera*, while the latter excludes such conditions as internal hydrocephalus. (Cerebral œdema may then be defined as cerebral swelling not due to an excess of intravascular blood.)

Cerebral shrinking may best be defined as a reduction of the brain volume below 92% of the cranial capacity, not due to localized loss of tissue (as, for example, porencephaly or post-traumatic cyst formation). These definitions refer, of course, only to physical criteria, which are sufficient for our purpose. There are no parallels to the chemical and histological criteria of cerebral œdema, since shrinking, like swelling, is a purely physical concept.

Cerebral shrinking has been defined in terms of a generalized process, but it may also occur in a localized form, as may cerebral swelling and œdema.

INCIDENCE AND ÆTIOLOGY

The condition may arise in either of two ways—(i) by cerebral vasoconstriction, or (ii) by cerebral dehydration.

The occurrence of cerebral vasoconstriction is not now to be doubted, and it is usually associated with general arterial hypotension. Although "surgical shock" is usually minimal in the case of head injury, the patient is often in a state of collapse at first. Lumbar puncture will often then reveal a low pressure. Surgical exploration in this early post-traumatic state not uncommonly exposes a low-tension brain which recedes from the craniotomy opening. Thus the intracranial pressure is sub-atmospheric (as it is in the normal erect person), and the cerebral volume, or less likely (for there is copious fluid) the amount of cerebro-spinal fluid, is diminished.

Experimental confirmation of the intracranial hypotensive effect of vasoconstriction has been obtained. Mechanical methods of "hypotensive anæsthesia" (such as venesection, or posturing the patient with head raised, as opposed to pharmacological methods) are effective because of passive narrowing of the vessels—particularly of the veins. Thus the intracranial pressure is lowered and a relatively "dry field" for surgery is obtained, but the minute volume of the cerebral circulation is little affected.

It has been suggested that general dehydration and arterial hypotension may account for the post-operative intracranial hypotension syndrome. Further, although the efficacy of planned dehydration therapy in reducing cerebral swelling is notoriously variable, a good

[1] Received on July 2, 1954.

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effect is sometimes obtained and may be due to reduction of intracranial plethora rather than to relief of œdema.

Cerebral dehydration — the other cause of cerebral shrinking—may occur in generalized form, as when the brain merely takes part in a general dehydration. As such, it may explain some cases of the hypotension syndrome, and is a readily corrected cause of post-operative drowsiness when apathy or stupor limits the fluid intake.

Of much greater interest is localized cerebral dehydration, for upon it depends the accommodation of a space-occupying lesion within the cranial cavity. A lesion such as a meningioma may reach a considerable size without any significant rise of intracranial pressure. This implies a reduction in volume of some other intracranial content. The concept of the incompressibility of normal brain—which is virtually a liquid—has been established only against strong opposition, based mainly upon confusion of compression and distortion. But though readily distorted, normal brain is very incompressible. It is therefore argued that, since brain is incompressible, tumours must be accommodated by reduction of the cerebro-spinal fluid or blood content of the cranium.

This reasoning is not acceptable. That the cerebro-spinal fluid volume is decreased remains to be proven, and the space that could be so provided is slight (150 millilitres at most in the average case even if all cerebro-spinal fluid was absorbed). Reduction of blood content is stated to occur because the rising intracranial pressure expresses blood by compressing the vessels. This would provide a very poor mechanism of compensation of pressure rise, since severe ischæmia would soon occur. Also, compression of the vessels will be effective first at the point of lowest pressure, which is the point of venous outflow—the vein-sinus junction—as shown by Wright (1938). (This is based upon the consideration that pressure is transmitted equally in all directions through the fluid brain, at least in each craniodural compartment, so that a pressure rise anywhere will first affect the veins.) The effect of compressing the vessels will therefore be to impede the outflow of blood rather than to increase it. Rising intracranial pressure does not narrow the vascular bed and so provide more space, but causes venous engorgement and increases the cranial content. How then is space provided?

Study of a pathological specimen will reveal the means of accommodation of a tumour. First, there may be some degree of cerebral shift. This may be quite gross even without much pressure rise, though the pressure is usually raised when tentorial herniation occurs. Secondly, the tumour appears to have replaced the brain tissue—as indeed it may have done in the case of a glioma. But the portion of a hemisphere in juxtaposition to a meningioma is indented and shrunken, and this loss of volume is not of essential nervous elements (as it is in hydrocephalus), since it may not prejudice normal function or complete recovery after the tumour has been removed. This situation is readily imitated with a fresh brain secured at autopsy. If a finger is pressed into its surface, it is readily accommodated—by the expression of tissue fluid. This occurrence is not an example of cerebral compressibility in the usual sense, for here the brain is dehydrated by pressure. The doctrine of incompressibility refers to brain which retains its normal cellular and fluid content.

To return to the phenomenon of cerebral shift, we may ask how herniated brain is accommodated in other cranio-dural compartments. A common autopsy finding in cases of cerebral glioma is that the tumour-containing hemisphere is grossly swollen. It may be even one and a half times the size of the other hemisphere. If the other hemisphere is considered normal, this would represent a 25% increase in brain bulk, which is obviously impossible within the rigid skull. Therefore we must conclude that swelling of one hemisphere is accommodated by shrinking of the other. Thus great cerebral shift may occur without excessive pressure rise—a frequent clinical finding.

Similarly, the hemisphere underlying a chronic subdural hæmatoma—often a low-pressure lesion—may be shrunken, and may transgress the mid-line only slightly. Such a hemisphere constitutes a problem for the surgeon, for it frequently fails to "expand" when the hæmatoma is evacuated, a "dead space" being left, into which recurrent hæmorrhage may take place, and providing the basis of the hypotension syndrome. This failure to expand is only to be expected, for the hemisphere is not displaced by high pressure so that it will respond immediately to decompression, but it is dehydrated and shrunken. Treatment by the injection, through a lumbar puncture, of warmed sterile Ringer's solution is a logical procedure, for it obliterates the dead space by bringing the brain up to the skull surface, achieves normal pressure, and reduces tentorial herniation.

These are but a few of the more familiar of many examples of accommodation of space-occupying lesions by cerebral shrinking, due to local dehydration. The following cases will illustrate the condition.

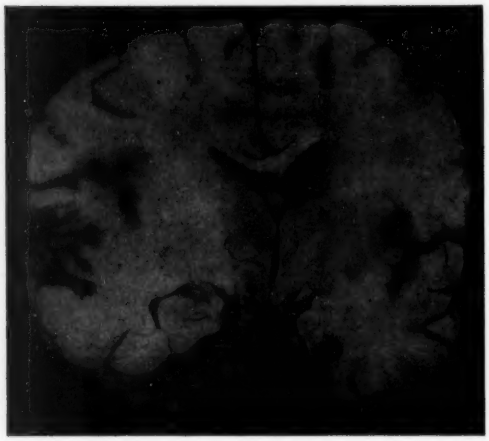

FIGURE I

Photograph of a coronal section of the brain in Case I, showing gross swelling of the right hemisphere from which a large tumour had been removed, in contrast to the left, in which a tumour may be seen to be still present

REPORTS OF CASES

CASE I.—A man, aged sixty-five years, complained of failing memory of six months' duration, and of left hemiplegia for two weeks, when he was admitted to hospital on July 28, 1953. He was then conscious though disorientated, but over the next two days he rapidly became comatose. Cerebral angiography showed a large vascular right temporal tumour, and this was excised at craniotomy on August 1. Tracheotomy was performed to treat aspiration pneumonia, but death occurred the next day.

Figure I is a photograph of the autopsy specimen. There was some tentorial and callosal herniation, and herniation of the cerebellar tonsils. The right temporal tumour had been completely excised, but there were two further tumours in the left cerebral hemisphere, and three in the cerebellum—all much smaller than the right temporal tumour had been. The primary tumour was a carcinoma of the lung. The photograph shows well the great swelling of the right cerebral hemisphere, despite complete excision of the tumour on that side, in contrast to the left hemisphere, which still contained two (smaller) tumours. The total cross-sectional area of the brain is relatively normal, being limited by the skull. The swelling of the right hemisphere was accommodated by shrinking of the left.

The rapid deterioration of consciousness occurred whilst the patient lay supine and became cyanosed because of aspiration pneumonia. Removal of a large tumour did not relieve the cerebral swelling.

CASE II.—A man, aged fifty-one years, presented a history of increasing dysphasia for three weeks, and right hemiplegia, maximal in the face and hand, for a few days. Severe headache and papillœdema were evidence of raised intracranial pressure. Cerebral angiography revealed a large space-occupying lesion in the left fronto-parietal region, biopsy of which revealed it to be a *glioblastoma multiforme*. Death occurred after a few weeks.

Figure II is a photograph of the autopsy specimen. The left cerebral hemisphere had herniated at the operation site, and to the right beneath the falx, but there was no tentorial herniation. The photograph shows the gross increase in bulk of the left cerebral hemisphere, and obvious shrinking of the right.

CASE III.—A woman, aged sixty-three years, was admitted to hospital six weeks after a minor head injury. For ten days she had suffered frontal headache

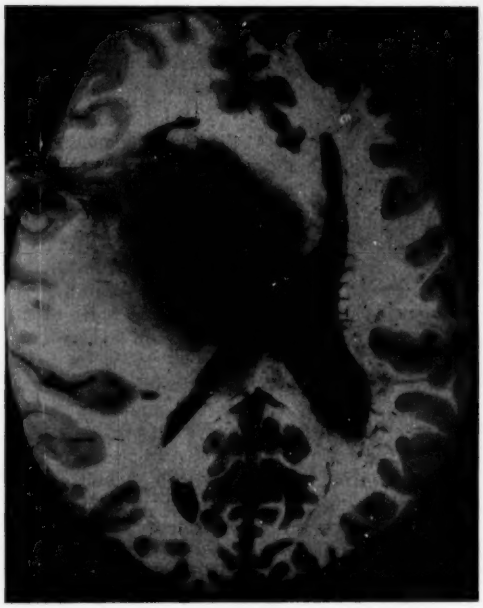

FIGURE II

Photograph of a horizontal section of the brain in Case II, showing increased bulk of the left cerebral hemisphere (with herniation at the biopsy site), in contrast to shrinking of the right hemisphere

and occasional vomiting, her state of consciousness had fluctuated daily, and she had a right hemiplegia. Her pupils were of normal size and reaction to light. Lumbar puncture showed the cerebro-spinal fluid to be under a pressure of 60 millimetres, and the fluid was straw-coloured.

At operation, a left chronic subdural hæmatoma was found. It was four centimetres deep and of fluid consistency, and covered the whole convexity of the

hemisphere. Its removal left a cavity measuring 20 by 10 by 3·5 centimetres (as measured at the temporal craniotomy site), into which the brain did not expand; 220 millilitres of Ringer's solution injected through a lumbar puncture obliterated this dead space and resulted in a pressure of 150 millimetres of cerebro-spinal fluid. Her recovery was uneventful.

This patient had a huge space-occupying lesion, but normal intracranial pressure and no pupillary evidence of tentorial herniation. The large volume of Ringer's solution required to obliterate the dead space is an indication of the degree of cerebral shrinking that had occurred.

MECHANISM OF LOCAL DEHYDRATION

The growth of a space-occupying lesion will result in increasing intracranial pressure. The elasticity of the spinal theca is such that an increase of volume of up to 1% of the total intracranio-spinal space is accommodated by displacement of cerebro-spinal fluid without much rise in pressure. But thereafter, unless a compensatory mechanism comes into action, the pressure will rise very steeply.

One important effect of pressure rise is that it disturbs the normal relationship between formation and absorption of tissue fluid. This relationship may be expressed by the familiar equation:

Tissue fluid pressure + blood osmotic pressure = capillary blood pressure + tissue fluid osmotic pressure.

Now for tissue fluid pressure we may write intracranial pressure, and we may take it that, at first, the osmotic pressures remain unchanged. Thus a rise of the intracranial pressure will tend to cause decreased formation and increased absorption of tissue fluid. Since the pressure of the blood in the cerebral capillaries and veins comfortably exceeds the intracranial pressure (for circulation continues and its pressure is supported by the vessels), slight rise of intracranial pressure will not raise the intravascular pressure.

Therefore a slight rise of intracranial pressure will result in cerebral dehydration, which will minimize continuing rise of the tissue fluid (intracranial) pressure. This is the mechanism of accommodation of space-occupying lesions without significant rise of intracranial pressure.

This balance is a precarious one, and the process has two apparent limiting factors. The first concerns the osmotic pressures, for dehydration might be expected to raise the tissue fluid osmotic pressure, which would tend to reverse the process. But as water is extracted, electrolyte balance will be restored by the extraction of electrolytes from the brain. This process obviously is limited, but it will allow considerable dehydration to occur. (Beyond this limit, there may occur loss of cellular constituents, as in the loss of white matter that occurs in hydrocephalus.)

The second, and more important limiting factor concerns the capillary blood pressure. We have already seen that the first effect of rising intracranial pressure upon the blood vessels is to impede venous outflow, resulting in venous congestion and rising venous and capillary pressure. Thus if more than slight rise of intracranial pressure occurs, the capillary blood pressure will be raised, and this will tend to terminate, or reverse, the dehydration process.

SIGNIFICANCE OF CEREBRAL SHRINKING

Let us now follow the course of an intracranial space-occupying lesion. If increase in size is slow, the initial small pressure rise is relatively limited, by the large dural septa, to the craniodural compartment concerned. This slight increase of pressure results in local dehydration, and the pressure rise is minimized. As the hemisphere is displaced, the pressure rise is communicated to other craniodural compartments, and here also the process of dehydration continues. Accommodation of the space-occupying lesion is thus adequate, if only temporary. At this stage there is very little general disturbance of the patient, although local dehydration may contribute to localizing signs.

At some stage, however, a critical transition point is reached, at which the intracranial pressure has risen sufficiently to embarrass venous outflow. From this point, the compensatory mechanism of dehydration in the primarily affected craniodural compartment is arrested, and then reversed. Increasing venous congestion raises the intracranial pressure yet further, and this in turn increases the embarrassment of venous outflow. Thus venous congestion is further increased, and local cerebral swelling is established. According to the exact relationship of capillary and intracranial pressure, cerebral œdema may also occur. Thus develops an increasing swelling in one craniodural compartment, whilst the brain in the other compartments, in which the pressure rise is less, continues to shrink. This may be the condition found at autopsy, or, if life permits, the same transition may occur throughout the brain, resulting in general cerebral swelling.

The clinical course of the patient reflects these changes. A large cerebral tumour may

have been present for years without causing much disturbance, but then quite rapidly there occurs deterioration of the clinical state. The growth of the tumour over this short period of deterioration may not be significant, but once the tenuous balance is upset, the process is a vicious-circle one of progressive deterioration, as illustrated by Cases I and II. The growth of a tumour is so slow that cerebral dehydration usually proceeds to its limit before the critical pressure is reached, so that once deterioration occurs, spontaneous improvement is rare.

In the case of a rapidly increasing lesion, such as an extradural hæmatoma, the opportunity for compensation by dehydration is much less, for the critical level of intracranial pressure is rapidly reached. Cerebral shrinking is then of little importance, but cerebral swelling and œdema, and much of the pressure rise, are still due to venous congestion.

The patient with a chronic subdural hæmatoma may have a similar course, but he classically exhibits daily fluctuations of consciousness, indicating that the vicious-circle mechanism is interrupted from time to time. The rate of enlargement of the hæmatoma so nearly approximates the rate of cerebral dehydration that the transition point may be temporarily overstepped, and then compensation may again proceed. With recumbency and respiratory depression at night, venous congestion and raised intracranial pressure occur, so that deterioration starts. If the patient is left lying in bed, the natural course of the lesion is seen to be progressive; but he is often only stuporose, and may be roused by his relatives or attendants. Then erect posture and stimulated respiration result in lower intracranial pressure, and his life depends upon such interruption of the vicious circle by environmental influences. His fluctuation of consciousness represents a fluctuation of his intracranial pressure about the critical point. On his better days he is compensating by dehydration; on his bad days he is decompensated with venous congestion.

Venous congestion is thus seen to be a state of the gravest significance. Cerebral œdema and cerebral swelling are not peculiar spontaneous occurrences, inexplicably due to the presence of an intracranial lesion, but are due to venous congestion. Even in the absence of any space-occupying lesion, a sustained rise of intracranial pressure above the critical level, or the occurrence of venous congestion, may initiate the same vicious circle. The most potent cause of venous congestion is carbon dioxide excess, which may be due to respiratory obstruction, or to pulmonary complications, or to respiratory depression (which occurs in direct proportion to the degree of unconsciousness). But this subject is beyond the scope of the present paper.

THERAPEUTIC PRINCIPLES

Cerebral shrinking is the compensatory mechanism which provides accommodation for space-occupying lesions. Thus until the lesion can be removed, cerebral shrinking must be maintained. To do this, one must prevent the intracranial pressure from exceeding the critical level, and combat any other cause of venous congestion. The following principles may be enumerated:

1. The intracranial pressure is lowest when the patient is erect, and is increased by recumbency. The patient should, if possible, be nursed sitting up, or should be encouraged to move about the ward if appropriate.

2. As a corollary, the patient should never be nursed with the head dependent, as is sometimes done to secure free drainage from the mouth, or to combat "shock", or to "arrest hæmorrhage by raising the intracranial pressure", or to "prevent cerebral ischæmia". If hæmorrhage is arterial in origin, the intracranial pressure will not arrest it, while if it is venous, dependency of the head results in venous congestion and further hæmorrhage. The notion that dependency of the head supports a failing circulation in these cases is open to question. In the comparable case of a limb in plaster of Paris, any evidence of circulatory embarrassment is treated by elevation of the limb, followed by decompression (splitting of the plaster cast) if necessary.

3. To prevent cerebral swelling due to venous congestion, hypercapnia must be avoided. Respiratory obstruction readily occurs in the unconscious patient, as does respiratory depression. The stuporose patient must not be allowed to lie supine, with stertorous and increasingly inefficient respiration, but must be stimulated and moved about. Oxygen administration is an inadequate method of treatment, for hypercapnia is not thereby relieved, and venous congestion is not reduced. Similarly, no respiratory depressant drug—such as a general anæsthetic—should be given until surgical relief of the lesion is undertaken.

4. The treatment in the advanced case centres upon the removal of the lesion, but this may form the minority only of the total amount of swelling present. The situation must be visualized, and dealt with, *in toto*.

5. Dehydration therapy may be a logical treatment of œdema, but I would prefer to direct treatment to the causative venous congestion.

SUMMARY

Cerebral swelling and cerebral œdema have been extensively studied, but the significance, and even the very occurrence, of cerebral shrinking have not been generally appreciated.

Cerebral shrinking by dehydration is the method of accommodation of slowly growing space-occupying lesions within the cranial cavity without excessive rise in pressure.

There is a critical transition point between cerebral shrinking and cerebral swelling, which is reached when the intracranial pressure is sufficient to obstruct venous outflow from the cranium. Cerebral venous congestion then results, and initiates a vicious-circle mechanism of deterioration.

Dependency of the head, and hypercapnia due to respiratory obstruction and depression, are potent causes of raised intracranial pressure and cerebral venous congestion, and are to be rigorously avoided by correct management.

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Dominion and State Committees

The Dominion and State Committees for the period 1954–1956 are as follows:

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General

Annie B. Cunning Lecture on Nutrition, 1954. Dr. F. W. Clements will deliver the Annie B. Cunning Lecture on Nutrition in the Stawell Hall of the College in Sydney on November 24, 1954, under the title "The Teeth and Food".

Gifts to Historical Library. The College has recently received from London a gift of valuable historical books from the Royal College of Physicians through its President, Sir Russell Brain, Bart. These books have come from the Harveian Library, and we are deeply indebted not only to the President, but also to Sir Edward Dodds, Harveian Librarian, for this magnificent contribution to our Historical Library. The gift is a further indication of the ties that bind our two Colleges in a common purpose. These books were displayed on October 14, 1954, at a meeting of the College in Sydney by the Curator of the Historical Library, Professor E. Ford.

Annual Meeting, 1955. The annual meeting in 1955 will be held in Sydney from Wednesday to Saturday, May 11 to 14.

Corrigendum

Dr. D. O. Shiels writes, in reference to his paper entitled "Elimination of Lead in Sweat", appearing in Volume 3, No. 3, 1954, at page 225, that the following errata occurred in the original typescript:

Table I, page 227, Column 1, subjects Y and C: *omit* the word "daily". In Column 8, the heading should be "Lead Excreted per Day in Sweat on Pad (Milligrammes)".

Table II, page 228, Column 6: The heading should read "Lead in Sweat on Pad (Milligrammes)".

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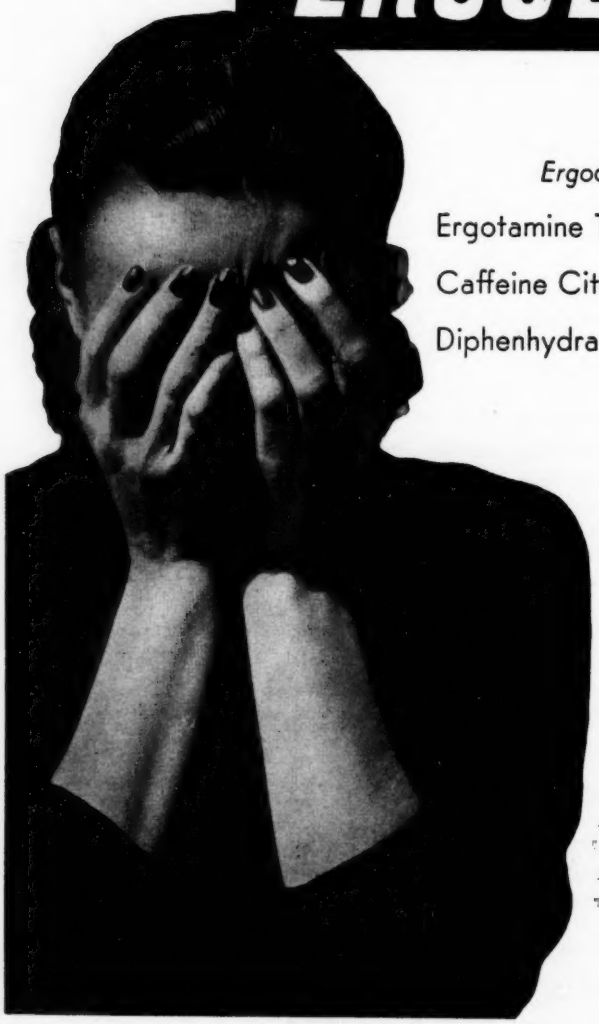

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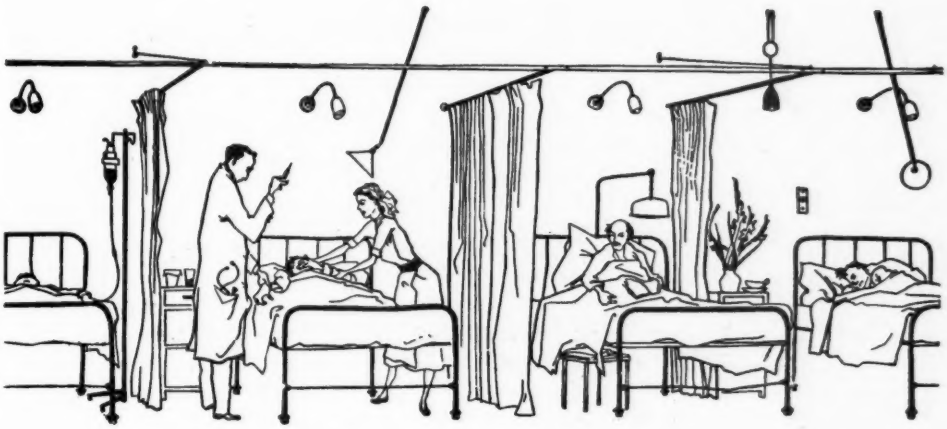

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